PHENOLOGICAL LITERACY

UNDERSTANDING THROUGH SCIENCE & STEWARDSHIP

Seminar Module 2

Back to the Future: the Use of Historical Records in Phenological Research

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Goals For Student Learning

This seminar module was created to help students:

- Appreciate the usefulness of historical data in reconstructing phenological patterns of the past
- Understand the types of phenological measurements that may be recorded using herbarium specimens
- Understand how researchers compare historical phenological observations with observations made in the present-day
- Learn how historical data can assist us in making predictions about the future

Historical Records And Phenology

Historical records and museum specimens have played important roles in documenting species' phenological responses to climate change. Contemporary studies generally vary in their duration and occur on relatively short time scales. Historical records and herbarium specimens, however, often date back to the past century. Miller-Rushing and Primack (2008) made use of phenological data collected by the American naturalist Henry David Thoreau and the botanist Alfred Hosmer**.** Miller Rushing and Primack (2008) evaluated the relationship between spring temperatures and flowering time in a plant community in Concord, Massachusetts. Their study demonstrated that: (1) on average plants are flowering earlier than they did 150 years ago, and (2) this shift to earlier flowering is associated with warming temperatures. Whether the warming temperatures are due to climate change or to a "heat-island" effect associated with urbanization is a topic that students should be encouraged to debate.

Two features of **herbarium** specimens make them amenable to phenological research. First, when feasible, botanists try to collect *whole plant specimens* for preservation in **herbaria**. Second, herbarium specimens include detailed information regarding the date and location of collection. Thus, for a given date and location, contemporary researchers can record detailed phenological measurements, such as: the date of first flowering (if a plant specimen bears only one flower, several buds, and no fruits) and the date of peak flowering (when, for example, 50% - 75% of a plant's buds have bloomed).

Primack et al. (2004) published one of the first studies to use herbarium specimens for the express purpose of linking past and current phenological patterns to climate change. Several other studies have been published in recent years, including a study by Robbirt et al. (2011), who used both field observations and herbarium specimens to evaluate long-term phenological patterns in a European orchid species (*Ophrys sphegodes*). Collectively, these studies suggest that plant phenological schedules have shifted in response to global climate change, and that herbarium specimens can be used to demonstrate this phenological change.

Articles To Read

Miller-Rushing, A. J., and R. B. Primack. 2008. Global warming and flowering times in Thoreau's concord: A community perspective. Ecology 89:332-341.

Primack, D., C. Imbres, R. B. Primack, A. J. Miller-Rushing, and P. Del Tredici. 2004. Herbarium specimens demonstrate earlier flowering times in response to warming in Boston. American Journal of Botany 91:1260-1264.

Robbirt, K. M., A. J. Davy, M. J. Hutchings, and D. L. Roberts. 2011. Validation of biological collections as a source of phenological data for use in climate change studies: a case study with the orchid *Ophrys sphegodes*. Journal of Ecology 99:235-241.

Suggested Discussion Questions

1. What are some benefits of using historical records to study phenology? Can you think of any specific challenges involved in conducting historical research? (Primack et al. 2004, Miller-Rushing and Primack 2008, Robbirt et al. 2011)

2. What are the main findings presented in Figure 2 in Miller-Rushing and Primack (2008)? What does each data point in Figure 2A represent? What does each data point in Figure 2B represent?

3. How did Primack et al. (2004) quantitatively estimate changes in flowering time and relate them to changes in Boston's spring temperatures?

4. What do the patterns presented in Figures 2 and 3 of Primack et al. (2004) suggest about the relationship between temperature and flowering time at the Arnold Arboretum? What are some factors that may have contributed to the strong relationship between temperature and flowering time observed in this study?

5. When combining data derived from multiple sources, it is important to reduce inconsistencies among sources that might compromise the data's ability to support the conclusions of a given study. This issue seems particularly relevant for Miller-Rushing and Primack's (2008) study, which combines data collected by three different research teams whose work spanned over 150 years. What steps did Miller-Rushing and Primack take to maximize consistency among observers?

6. Over how many years did the herbarium data analyzed by Robbirt et al. (2011) span? Over how many years did the field phenological observations analyzed by Robbirt et al. (2011) span? What was Robbirt et al.'s (2001) motivation for comparing phenological measurements obtained from field observations to those obtained from herbarium specimens?

7. How (if at all) has the flowering phenology of *Ophrys sphegodes* changed over time? Upon what evidence from the paper do you base your opinion?

8. Do you think that Robbirt et al.'s (2011) study confirms that herbarium data collection is an appropriate substitute for field observations in *Ophrys sphegodes*? Upon what evidence from the paper do you base your opinion?

9. What do these papers as a whole suggest about the use of historical records/herbarium specimens in phenological research? Can you think of any instances where these types of legacy data might **not** be appropriate?

10. Henry David Thoreau and Alfred Hosner kept careful phenological records in their local neighborhoods. Has anyone in this seminar ever written down their natural history observations on a regular basis? Why or why not?

11. There are many other sources of historical phenological data. For example, the Japanese have been carefully recording the dates of annual cherry blossom festivals (or *Hanami*) since the mid-1400's! What are some other potential sources of historical phenological information that might enhance our understanding of long-term phenological patterns?

Glossary

- **Herbarium (**plural = **herbaria):** a collection of preserved and labeled plant specimens. Many herbaria house plant specimens that span a wide geographic area and that date back many years. Specimens are carefully archived and arranged so that they can be easily retrieved.

Note for Instructors

We have also developed an herbarium research activity for advanced undergraduate students that could be integrated into this seminar module. The activity includes a phenological data set generated from California poppy (*Eschscholzia californica*) herbarium specimens. The activity also includes instructions for preparing, visualizing, and statistically analyzing the herbarium data so that students can determine whether long-term changes in flowering phenology have occurred. The herbarium research activity can be downloaded at: www.usanpn.org.

GLOBAL WARMING AND FLOWERING TIMES IN THOREAU'S CONCORD: A COMMUNITY PERSPECTIVE

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Abstract. As a result of climate change, many plants are now flowering measurably earlier than they did in the past. However, some species' flowering times have changed much more than others. Data at the community level can clarify the variation in flowering responses to climate change. In order to determine how North American species' flowering times respond to climate, we analyzed a series of previously unstudied records of the dates of first flowering for over 500 plant taxa in Concord, Massachusetts, USA. These records began with six years of observations by the famous naturalist Henry David Thoreau from 1852 to 1858, continued with 16 years of observations by the botanist Alfred Hosmer in 1878 and 1888–1902, and concluded with our own observations in 2004, 2005, and 2006. From 1852 through 2006, Concord warmed by 2.4°C due to global climate change and urbanization. Using a subset of 43 common species, we determined that plants are now flowering seven days earlier on average than they did in Thoreau's times. Plant flowering times were most correlated with mean temperatures in the one or two months just before flowering and were also correlated with January temperatures. Summer-flowering species showed more interannual variation in flowering time than did spring-flowering species, but the flowering times of spring-flowering species correlated more strongly to mean monthly temperatures. In many cases, such as within the genera *Betula* and *Solidago*, closely related, co-occurring species responded to climate very differently from one another. The differences in flowering responses to warming could affect relationships in plant communities as warming continues. Common St. John's wort (*Hypericum perforatum*) and highbush blueberry (*Vaccinium corymbosum*) are particularly responsive to changes in climate, are common across much of the United States, and could serve as indicators of biological responses to climate change. We discuss the need for researchers to be aware, when using data sets involving multiple observers, of how varying methodologies, sample sizes, and sampling intensities affect the results. Finally, we emphasize the importance of using historical observations, like those of Thoreau and Hosmer, as sources of long-term data and to increase public awareness of biological responses to climate change.

Key words: climate change; Concord, Massachusetts; flowering times; global warming; Henry David Thoreau; phenology.

INTRODUCTION

It is astonishing how soon and unexpectedly flowers appear, when the fields are scarcely tinged with green. Yesterday, for instance, you observed only the radical leaves of some plants; to-day you pluck a flower.

—Henry David Thoreau (Thoreau 1962)

Climate change is already affecting biological systems worldwide (Walther et al. 2002, Parmesan and Yohe 2003, Root et al. 2003). Several studies have detected effects of climate change on changes in species distributions (Grabherr et al. 1994, Parmesan et al. 1999), rates of extinctions (McLaughlin et al. 2002, Pounds et al.

2006), the storage of carbon in plants and soils (Shaver et al. 2000), and the timing of life history or phenological events (Menzel and Fabian 1999, Inouye et al. 2000, 2003, Primack et al. 2004). Of these biological responses to climate change, changes in the timing of phenological events are the most widely reported and probably the most easily detectable (Parmesan and Yohe 2003, Root et al. 2003). Climate-related changes in phenology, some quite dramatic, have been observed on every continent and in the oceans (e.g., Menzel and Fabian 1999, Inouye et al. 2000, Schwartz and Chen 2002, Edwards and Richardson 2004, Gordo et al. 2005, Barbraud and Weimerskirch 2006, Beaumont et al. 2006).

In most instances, phenological events, such as flowering, bird migration, and amphibian reproduction, are now occurring earlier than in the past (Parmesan and Yohe 2003, Root et al. 2003). However, it is clear that species' phenologies are changing at different rates. In some cases, different phenological events are changing at different rates even within a single species or individual plant or animal (Post et al. 2008). These changes have

the potential to alter relationships among many species (Stenseth and Mysterud 2002, Visser and Both 2005) and could alter species' exposures to abiotic factors such as frost (Inouye 2008). Some changes in intertrophic interactions are already evident (Inouye et al. 2000, Edwards and Richardson 2004, Both et al. 2006). For plants, studies have shown significant variation in the rates at which flowering times are changing across species (Fitter et al. 1995, Bradley et al. 1999, Sparks et al. 2000). For example, in England, some species are flowering more than a month earlier than they did 50 years ago, while other species' flowering times are not changing (Fitter et al. 1995). From these findings arise several questions: Why do species respond differently to climate change? Can we better characterize these differences? What species, or groups of species, are most (or least) sensitive to changes in climate? More specifically, does season of flowering or growth form explain any of the variation we see in the responses of individual species to variations in climate?

The answers to these questions could impact on individual performance (Gross and Werner 1983, Parra-Tabla and Vargas 2004) and population and community dynamics (Inouye et al. 2000, Visser and Both 2005). Rare and endangered species that do not adapt to these changes could face extinction. Unfortunately, the number of species included in most historical data sets has limited previous studies. To our knowledge, only one major study has examined the responses of enough plant species (in this case, 243) to search for biological patterns that might explain differences in species' responses (Fitter et al. 1995, Fitter and Fitter 2002). That study showed that 16% of species flowered significantly earlier in the 1990s than in previous decades. Early-flowering species, annuals, and insect-pollinated species showed the greatest sensitivity to climate change (Fitter and Fitter 2002). Although their findings are significant, the observed trends may be valid only for Europe or central England, where the study took place.

The purpose of our study was to characterize how flowering times respond to variations in climate in North America. To this end, we analyzed data from a previously unstudied record of first flowering dates in Concord, Massachusetts, USA. Our data set is unique in several aspects. First, it spans an exceptionally long period of time—155 years from 1852 to 2006—which we accomplish by combining three individual sets of observations. Second, our data set includes observations on over 500 plant taxa, which allows us to identify patterns that occur at the community level. Finally, our data set begins with the observations of Henry David Thoreau, the famous naturalist, philosopher, and author of the widely read book *Walden*, which could make these results particularly relevant to a nonscientist audience. With this unique set of data, we investigated abiotic factors contributing to variation in flowering responses to climate change. We tested the hypothesis that climate change has altered phenology, and we identified potential mechanisms responsible for these phenological changes.

METHODS

Since the 1850s, several botanists have recorded flowering times in Concord, Massachusetts, USA. These records began with the work of Henry David Thoreau, who observed the first flowering dates (FFD) of over 500 species of plants in Concord from 1852 to 1858 (Thoreau 1962; unpublished tables courtesy of B. P. Dean). Alfred Hosmer, a shopkeeper and amateur botanist, continued these observations of FFDs in Concord for over 700 plant taxa in 1878 and 1888–1902 (Hosmer 1878–1903). Thoreau's and Hosmer's records included the flowering times of plants in all habitat types. Later, from 1963 to 1993, Pennie Logemann, a Concord landscape designer, maintained records of flowering times for over 250 species of plants that occurred on her property, which consisted primarily of forest and wetland. Each of these botanists observed new taxa in flower several days per week during the flowering season. Thoreau intended to write a book about phenology, but did not complete it before his death (Thoreau 1993, 1999). We do not know why Hosmer kept phenological records, as he never wrote any papers based on his observations other than those intended to update the flora of Concord (Hosmer 1899a, b). Logemann made phenological calendars as an aid for designing gardens. We know that each of these naturalists had a good working knowledge of the flora of Concord, because of their abilities to distinguish taxa that differ in subtle characteristics (Eaton 1974).

We made our own observations of flowering times in Concord from 2003 to 2006. We purposefully used methods similar to those of the previous naturalists, particularly Thoreau and Hosmer. Two or three days a week from March to October, we recorded plants in flower across Concord. We observed over 500 species in flower. For the analyses in this study, we did not use the observations we made in 2003, because at that time we were still learning the locations of the plants in Concord and frequently missed the earliest flowering dates.

We analyzed in detail the FFDs for 43 common, early-flowering species for which we had the most flowering data. For these species, we included observations made by Thoreau (six years, 1852–1858), Hosmer (16 years, 1878 and 1888–1902), and ourselves (three years, 2004–2006), for a total of 25 years of observations. For each species, we had FFD data for at least 19 of the 25 years. Because we did not have observations for each species in each year, we calculated the difference between the FFD in each year and the FFD in the benchmark year of 1893, a year for which we had observations for all 43 species. This calculation minimized biases caused by different species missing from each year. We used regression analysis to determine the relationship between FFD and mean monthly temper-
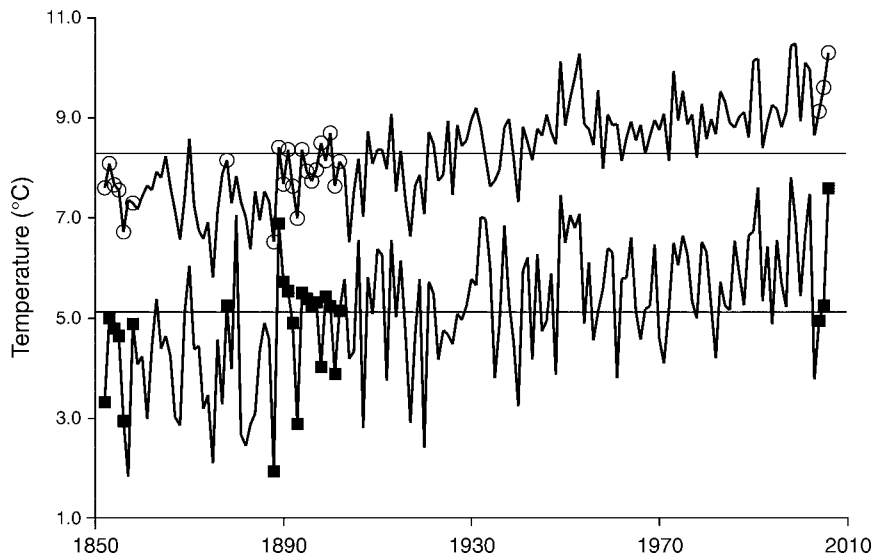
FIG. 1.   Temperatures at Blue Hill Meteorological Observatory (33 km southeast of Concord, Massachusetts, USA) from 1852 to 2006. The upper line and open circles represent mean annual temperatures. The lower line and solid squares represent mean monthly temperatures in January, April, and May, temperatures that were highly correlated with flowering times for many species. Horizontal lines show long-term means for each (annual = 8.3°C; Jan, Apr, May = 5.1°C). Circles and squares show years with flowering data.

atures. Logemann observed first flowering dates for eight of these species in at least nine years during the period 1963–1993. We included her observations in regressions for these eight species.

For a broad survey of flowering phenology, we analyzed the records of the 296 taxa (293 species, three distinct subspecies) of flowering plants for which Hosmer had made an observation in each of 15 years, 1888–1902. We compared FFD in each year with mean monthly temperatures. For each taxon, we correlated the FFD with the mean monthly temperatures of the month of flowering and each of the 11 preceding months. From those correlations, we found the months for which the mean temperatures were best correlated with FFD. We also correlated each FFD with the mean temperature for January and the two months preceding flowering, as temperatures in those months were often significantly correlated with FFD. Following the example of Fitter et al. (1995), we used standard deviations about the mean FFD as a measure of interannual variation in flowering time. We then used the regression analyses and standard deviations to compare several groups of taxa—e.g., plants that flower in different months; native and nonnative taxa; and annuals, perennial herbs, and woody plants—in order to find patterns that might explain the overall variation in response to year-to-year changes in climate.

For our analysis, we used dry bulb air temperatures recorded in a standardized way at Blue Hill Meteorological Observatory in Milton, Massachusetts, USA (33 km southeast of Concord). Unfortunately, the weather records for Concord were not complete for the time period between 1888 and 1902. However, we correlated

the available Concord temperature records (1931–1949) with those for Blue Hill Observatory and found that mean monthly temperatures for each year had a correlation coefficient of 0.995 or higher. Thus, we are confident that the temperature in Concord was closely related to that at Blue Hill Observatory.

RESULTS

For 43 common, spring-flowering species (33 native, 10 nonnative), we combined 25 years of observations by three different observers (Thoreau, Hosmer, and ourselves) that span the years 1852–2006. Over this time, mean annual temperatures in Concord rose by 2.4°C and mean monthly temperatures in January, April, and May rose by 2.3°C, as determined by linear regression (Fig. 1). Our analysis of these observations showed that these plants have flowered progressively earlier over the past 150 years (Fig. 2a). For the 43 species, the mean FFD during Thoreau's observations (1852–1858) was 14 May, whereas the mean FFD for our observations (2004–2006) was 7 May, seven days earlier. The mean FFD for Hosmer (1878, 1888–1902) was 10 May, intermediate between Thoreau's and our own observations. The differences in FFDs among the three time periods were highly significant as determined by two-way ANOVA, considering time period (Thoreau, Hosmer, and ourselves) and species as factors ($P < 0.001$). The FFD for some species changed dramatically from 1852 to 2006. For example, highbush blueberry (*Vaccinium corymbosum*), a native shrub, and yellow wood sorrel (*Oxalis europaea*), a native herb, are now flowering 21 and 32 days earlier than they did 150 years ago, respectively.

FIG. 2. Change in mean first flowering dates (FFD) for 33 native and 10 nonnative species (a) over time and (b) in response to warming mean monthly temperatures in January, April, and May. (a) Symbols correspond to observers and time periods: solid squares for observations by Henry David Thoreau (1852–1858), open triangles for observations by Alfred Hosmer (1878, 1888–1902), and solid circles for our observations (2004–2006). Solid horizontal bars with standard error bars represent the mean FFD for each observer. Each point (other than solid bars) was calculated by using the difference between when a species flowered in a particular year and when it flowered in the benchmark year of 1893, when all species were observed. Then we averaged these differences among species; each point represents the mean difference in FFD from 1893 for all species observed in a particular year. (b) Solid diamonds and the solid line represent mean FFD for 33 native species. Open circles and the dashed line represent mean FFD for 10 nonnative species. Means were calculated as described for (a), as differences from FFD in 1893. Lines are best-fit regressions. Natives flowered 2.93 days earlier per 1°C warming ($R^2 = 0.609$, $P < 0.001$). Nonnatives flowered 3.40 days earlier per 1°C warming ($R^2 = 0.428$, $P < 0.001$).
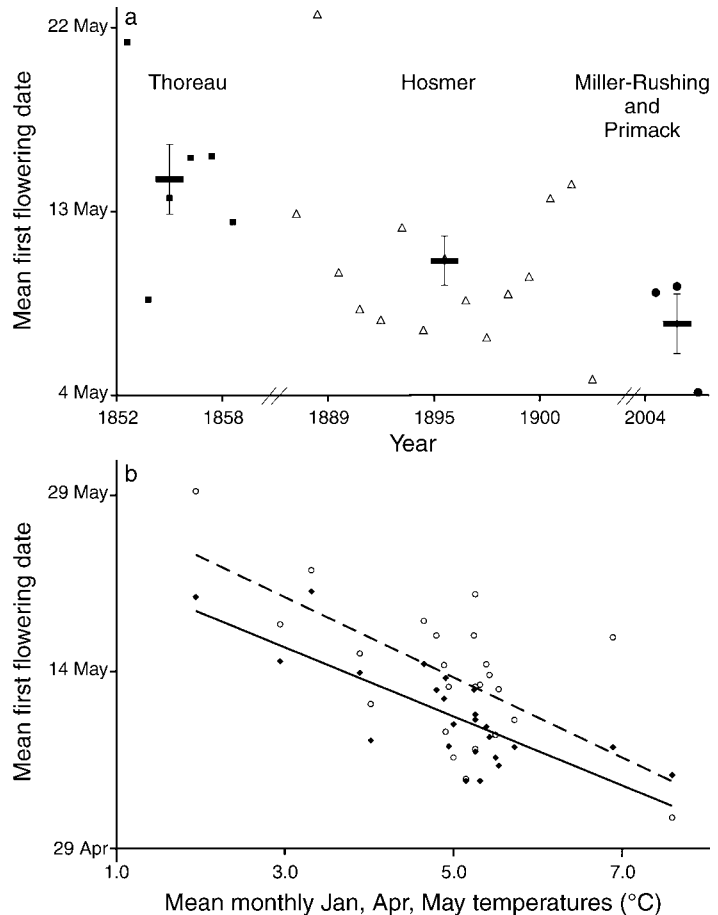
The earlier flowering times were strongly correlated with warming mean monthly temperatures in January, April, and May over that time period. On average, plants flowered 3.07 days earlier for each 1°C increase in mean monthly temperatures, as determined by linear regression (43 species, $R^2 = 0.609$, $P < 0.001$; Fig. 2b). The changes in FFD for native (33 species, 2.93 days earlier per 1°C, $R^2 = 0.596$, $P < 0.001$) and nonnative (10 species, 3.40 days earlier per 1°C, $R^2 = 0.428$, $P < 0.001$) were virtually identical. Average January, April, and May temperatures were 4.3°C during Thoreau's observations, 5.0°C during Hosmer's observations, and 5.9°C during our own observations.

Of these 43 common species, Logemann observed eight, all native, in at least nine years (1963–1993). Inclusion of her observations improved the ability of temperature to explain FFDs for three species—shadbush (*Amelanchier canadensis*), bunchberry (*Cornus canadensis*), and wild strawberry (*Fragaria virginiana*)—as indicated by R^2 values from the flowering-temperature relationship; R^2 values increased when her observations were included. FFDs of three species were not correlated with temperature, with or without Logemann's observations, and Logemann's observations did

not improve the explanatory power for the remaining two species.

For our broad survey of 296 species that Hosmer observed from 1888–1902, mean FFD ranged from 4 March, for skunk cabbage (*Symplocarpus foetidus*), to 14 August, for swamp rose mallow (*Hibiscus palustris*). Some species, such as witch hazel (*Hamamelis virginiana*), flowered earlier or later, but Hosmer's records for those species were not complete. Most plant taxa flowered in May (82), June (86), and July (76); fewer taxa flowered in March (2), April (32), and August (18); and the mean flowering date for all plants was 12 June.

On average, the Concord plants observed by Hosmer responded to each 1°C increase in mean January, April, and May temperatures by flowering 3.28 days earlier (296 taxa, $R^2 = 0.84$, $P < 0.001$). Of the 296 taxa we examined, 279 (94%) flowered earlier in years with warmer mean monthly temperatures in January and the two months prior to flowering, as indicated by negative correlations; 168 (57%) showed significant ($P < 0.05$) correlations between FFD and mean monthly temperatures. No taxon showed a significant trend toward later FFD with warmer mean monthly temperatures. For 20 taxa, mean monthly temperatures explained more than 60% of the variation in FFDs ($R^2 > 0.60$; Table 1). For

TABLE 1. The 20 taxa with first flowering dates (FFD) best predicted by mean monthly temperatures in January and the two months prior to flowering, out of a sample of 296 taxa.

Species	Common name	R^2	Change	Mean FFD	SD
Hypericum perforatum†	common St. John's wort	0.73	−3.5	21 Jun	5.1
Cichorium intybus†	chicory	0.70	−3.5	30 Jun	5.1
Amelanchier canadensis	shadbush	0.70	−3.4	2 May	5.6
Viola pubescens	downy yellow violet	0.69	−4.5	9 May	7.3
Vaccinium corymbosum	highbush blueberry	0.67	−5.6	8 May	9.3
Erigeron pulchellus	Robin's plantain	0.66	−5.8	21 May	9.6
Kalmia polifolia	pale laurel	0.66	−3.2	12 May	5.3
Chelidonium majus†	celandine	0.66	−4.3	13 May	7.2
Chamaedaphne calyculata	leatherleaf	0.65	−3.2	22 Apr	5.7
Melampyrum lineare	cowwheat	0.64	−3.4	16 Jun	5.2
Vaccinium vacillans	late low blueberry	0.64	−4.6	4 May	7.7
Lysimachia terrestris	swamp candles	0.64	−4.5	25 Jun	6.8
Maianthemum canadense	Canada mayflower	0.63	−3.4	18 May	5.8
Rhododendron nudiflorum	pink azalea	0.63	−4.5	26 May	7.7
Iris versicolor	larger blue flag	0.63	−4.0	1 Jun	6.1
Cornus canadensis	bunchberry	0.62	−4.4	22 May	7.6
Ranunculus bulbosus†	bulbous buttercups	0.62	−5.1	4 May	8.8
Viola cucullata	marsh blue violet	0.61	−3.2	27 Apr	5.8
Houstonia caerulea	bluets	0.61	−4.2	18 Apr	7.6
Ligustrum vulgare†	privet	0.60	−6.2	23 Jun	9.8

Notes: Change is given as days/°C. $P < 0.001$ for all taxa shown. Negative change indicates a change toward earlier FFD in warmer years.

† Nonnative species.

example, 73% of the variation in the FFD of common St. John's wort (*Hypericum perforatum*) was explained by changes in mean monthly temperatures in January, April, and May. Two other species sensitive to mean monthly temperatures, privet (*Ligustrum vulgare*) and robin's plantain (*Erigeron pulchellus*), each responded to each 1°C increase in temperatures by flowering about six days earlier.

Because taxa respond more to temperatures in individual months rather than annual temperatures (Fitter et al. 1995, Sparks and Carey 1995), we examined the relative importance of each month's mean temperature in predicting changes in mean FFD. Of the 221 taxa with FFDs that were significantly correlated with the mean temperature of at least one month ($P < 0.05$), 116 (52%) were correlated with mean May temperatures, while 100 (45%) were correlated with mean January temperatures (Fig. 3). The FFDs of 162 (73%) taxa were correlated with the mean temperatures either in the month of flowering or in one of the two months prior to
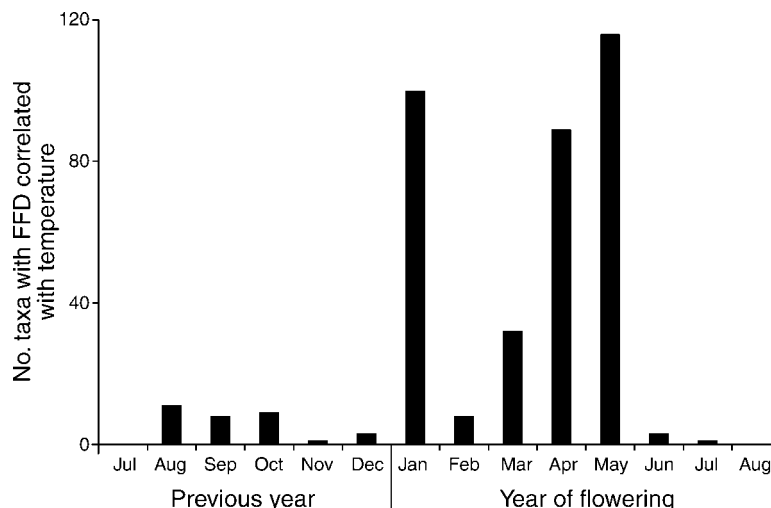

FIG. 3. Frequency with which each month's mean temperature was significantly correlated with the first flowering date (FFD) of a taxon. Only significant correlations are shown ($P < 0.05$). The pattern is consistent whether the number of taxa or the percentage of possible occurrences is considered. The FFDs for a total of 221 taxa were significantly correlated with mean temperatures in at least one month. We tested correlations between FFD and temperatures in the month of flowering and in the 11 preceding months. Months are shown as occurring during the year of flowering or during the previous calendar year.

flowering. Fig. 3 shows three peaks where months were relatively important in predicting FFD compared to nearby months: large peaks in April, May, and January of the flowering year. A small number of species had FFDs correlated with temperatures in August, September, and October of the year prior to flowering. This pattern, which is consistent whether absolute number of occurrences or percent of possible occurrences are considered (data not shown), suggests that many species are particularly sensitive to cold January temperatures and to warming temperatures early in the spring or just prior to flowering.

The peak in January is particularly striking, because FFDs were often significantly correlated with mean January temperature, but very rarely correlated with the adjacent months, i.e., December of the previous year and February of the flowering year. Mean January temperatures were significantly correlated with FFDs for equal percentages of all growth forms (approximately 33% each of annuals, perennial herbs, and woody shrubs). It is possible that these correlations were due to severely cold temperatures in January. January was the coldest month in eight out of the 15 years that Hosmer kept records (1888–1902).

Due to the large number of taxa included in our analysis, we were also able to examine the effects of season of flowering, growth form, nativeness, and habitat on responses to climate change. We found that FFDs for early-flowering (March, April, May, June) taxa were more correlated with mean monthly temperatures than were FFDs for late-flowering (July, August) taxa as shown by regression analysis (296 taxa, $P <$ 0.001; Fig. 4). In other words, the mean FFDs of early-flowering taxa were better predicted by and more responsive to mean monthly temperatures than were late-flowering taxa. Even though FFDs of early-flowering species were more correlated with temperature, we found that late-flowering taxa had greater standard deviations about their mean flowering dates than did early-flowering taxa, as determined by regression analysis ($P = 0.016$).

When we analyzed the same relationship according to growth form, we found that growth forms differed significantly in their patterns. The standard deviations of annuals were not significantly affected by season of flowering (18 taxa, $P = 0.120$); late-flowering perennial herbs had greater standard deviations than early-flowering taxa (194 taxa, $P < 0.001$). Woody plants, however, displayed an opposite trend: early-flowering taxa had greater standard deviations than late-flowering ones (66 taxa, $P = 0.032$). Because the majority of taxa in the data set were perennial herbs (65%), it is likely that the relationship between standard deviation and mean FFD for perennial herbs drove the trend seen when all taxa were considered together. In addition, on average, annuals showed a marginally significantly greater standard deviation about their mean FFDs than did perennial herbs (11.2 compared to 8.7 days, $t = 1.92$,
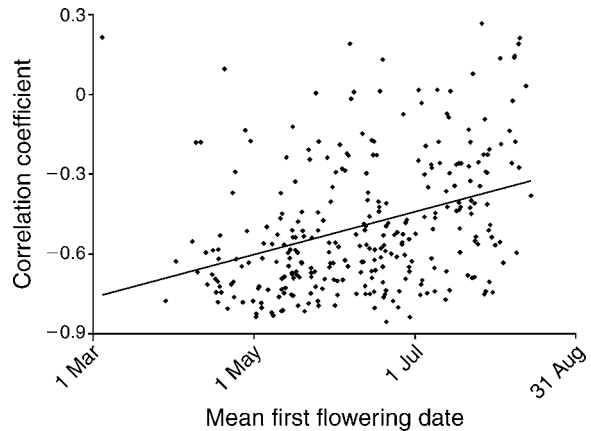

Fig. 4. The relationship between mean first flowering date (FFD) from 1888 to 1902 and the correlation between FFD and mean monthly temperatures in January and the two months prior to flowering for 296 plant taxa. Each point represents one taxon. Negative correlation coefficients indicate earlier flowering in warmer years. Slope = 0.003, $R^2 = 0.141$, $P < 0.001$.

two-tailed $P = 0.07$), which in turn showed a significantly greater standard deviation than did woody plants (8.7 compared to 7.4 days, $t = -2.77$, two-tailed $P = 006$). That is, life-form explained, in part, why some taxa had more year-to-year variation in flowering compared to others.

We found that nonnative taxa did not differ from native taxa in their flowering responses to temperature. Both native (239 taxa) and nonnative (54 taxa) taxa showed a great deal of variation of response, but neither standard deviations about mean FFDs (8.6 vs. 9.2 days, $t = -0.971$, two-tailed $P = 0.33$) nor correlations with mean monthly temperatures (correlation coefficients of -0.490 vs. -0.523, $t = 0.863$, two-tailed $P = 0.39$) differed significantly between the two groups. Similarly, habitat (aquatic, forest, grassland, roadside, wetland) did not explain any of the variation in flowering responses to temperature.

The FFDs of many closely related (i.e., within the same genus) and co-occurring species responded to changes in temperature at very different rates. For example, black birch (*Betula lenta*) and gray birch (*Betula populifolia*), which occur in many of the same habitats in Concord, show very different responses to temperature (Miller-Rushing and Primack, *in press*). Black birch flowered 2.83 days earlier for each 1°C increase in January, March, and April temperatures ($R^2 = 0.376$, $P = 0.015$), whereas gray birch FFDs showed no relationship with temperature ($P = 0.535$). In an even more dramatic example, rough-stemmed goldenrod (*Solidago rugosa*) flowered 11.17 days earlier for each 1°C increase in January, May, and June temperatures ($R^2 = 0.554$, $P = 0.001$), whereas the FFDs of lance-leaved goldenrod (*Solidago graminifolia*) and most other goldenrods showed no relationship with temperature ($P = 0.535$). Among the 52 genera for which Hosmer

observed more than one species, 31 (60%) contained at least one species with FFDs significantly correlated to mean monthly temperatures in January and the two months prior to flowering and at least one species with FFDs that were not correlated with temperature. Of the 25 genera for which Hosmer observed just two species, 10 (40%) contained one species with FFDs significantly correlated to mean monthly temperatures and one species with FFDs not correlated to temperature.

DISCUSSION

On average, plants in Concord appear to flower now seven days earlier than they did when Thoreau made his observations (1852–1858). Most of this change in flowering time is probably due to rising winter and spring temperatures. Temperatures in eastern Massachusetts have increased more rapidly than in many other areas of the world due to the combination of global warming and the urban heat island effect (New England Regional Assessment Group 2001). The rate at which Concord plants responded to warming—3.3 days earlier flowering for each 1°C increase in mean monthly temperatures in January, April, and May—fits well with findings in Europe (Sparks and Carey 1995, Chmielewski and Rötzer 2001, Fitter and Fitter 2002) and North America (Schwartz and Reiter 2000, Cayan et al. 2001).

We also found that mean monthly temperatures in January and the two months immediately preceding flowering were significantly correlated with the FFDs for many species. It is known that plants respond to temperatures from the previous fall (Fitter et al. 1995). Cooling temperatures in the fall and winter often contribute to the vernalization process, in which colder temperatures lead to increased competence and earlier flowering (Chuine 2000, Sung and Amasino 2004). However, we found that colder January temperatures were correlated to later flowering times. One reason for the discrepancy could be the difference between climate patterns in the northeastern United States and Western Europe, the site of many previous studies of plant phenology (e.g., Fitter et al. 1995, Sparks et al. 2000). Winter temperatures in Western Europe's maritime climate tend to be significantly milder than those in the continental climate of the northeastern United States (Hartmann 1994, Seager et al. 2002). For an average of 12 days in each January (1963–2006), there is no snow cover in Concord to insulate plants from the extreme cold. Thus, overwintering plants and seeds in the northeastern United States may be much more susceptible to damage from January's extreme cold temperatures than are plants in Western Europe. It is also possible that the phenologies of species in colder climates may simply be particularly sensitive to climate (Thórhallsdóttir 1998).

In another surprising finding, early-flowering perennial herbs had FFDs that displayed less interannual variability than did those of late-flowering taxa, although the variation in early-flowering taxa was more closely linked to mean monthly temperatures (Fig. 2). Previous studies have found individual examples of highly variable flowering times in late-flowering perennial herbs, such as bird's foot trefoil (*Lotus corniculatus*; Ollerton and Lack 1998), but we do not know of previous evidence suggesting that it may be a widespread pattern. Woody species showed the more usual pattern of greater interannual variation in flowering times for early-flowering species rather than late-flowering species (Fitter et al. 1995, Post and Stenseth 1999). It seems that the flowering times of many late-flowering perennial herbs may have been linked to an indicator, or set of indicators, that were more variable than mean monthly temperatures, or that late-flowering perennial herbs have inherently variable flowering times. Possibilities of non-temperature indicators for flowering times include phenomena such as rainfall, shading, and land use. It is also possible that monthly temperatures were too coarse to have a detectable effect on flowering times for these species, and that daily temperatures may be more appropriate. Further study is clearly necessary to isolate the factors responsible for the high variation in the flowering dates of late-flowering perennial herbs. Intriguingly, Rich et al. (2008) found additional differences between woody and herbaceous species in a piñon–juniper woodland suggesting that herbaceous species are more responsive to environmental variation than are woody species.

The flowering times of several species appear sensitive enough to changes in temperature that they could serve as indicator species and be used to measure biological responses to changes in climate over time. Among the species in our study, two particularly common species—common St. John's wort (*Hypericum perforatum*) and chicory (*Cichorium intybus*)—both had high correlations with mean monthly temperatures ($R^2 > 0.60$) and had mean FFDs that advanced more than three days per 1°C increase in temperature (Table 1). Although these species are nonnative, they are both common in urban and rural areas across the United States and easy to identify. Common and widespread native species, such as highbush blueberry (*Vaccinium corymbosum*), Canada mayflower (*Maianthemum canadense*), and larger blue flag (*Iris versicolor*; see Plate 1) could also serve as indicator species (Table 1). Before these species are utilized as indicator species across their ranges, however, we suggest that studies determine if their sensitivity to changes in temperatures is consistent across their ranges. Their usefulness as indicators for interacting species should also be tested, as Both et al. (2006) have shown that phenological changes may differ among different parts of a food chain.

Our study suggests that flowering times are changing at different rates for several closely related, co-occurring species, such as those within the genera *Betula* and *Solidago*. As the timing of flowering and other correlated life history traits change for these species, interactions among the species will also change. Un-

PLATE 1. The first flowering dates of larger blue flag (*Iris versicolor*) were highly correlated with changes in temperature in Concord, Massachusetts (USA). The flowering date of this species may provide a good indicator of biotic responses to climate change. Photo credit: A. J. Miller-Rushing and R. B. Primack.

doubtedly, these changes will be to the advantage of some species and disadvantage of others, although it is difficult to predict the winners and losers. It is also clear that the net effect of phenological changes on the fitness of individuals or species will depend on complex, timing-based interactions, sometimes spanning multiple trophic levels (Stenseth and Mysterud 2002, Visser and Both 2005). For example, plants with particularly rapid responses to changes in temperature could flower before the emergence of their pollinators, thus decreasing their chances of reproductive success (Kudo et al. 2008). In other cases, plants may become more susceptible to frost events or benefit from the lengthening of the growing season (Inouye 2008, Kudo et al. 2008). As studies like ours identify the species most sensitive to changes in climate, researchers can specifically include these species in their examinations of the ecological and evolutionary impacts of non-synchronous shifts in flowering times.

In many instances, the best long-term phenological data may contain observations made by several observers over long periods of time, as in our study. In these cases, researchers must be mindful of the different time periods and the methods that various observers might use, including sampling effort (days/week, hours/day, total area examined) and definitions of what constitutes an open flower. For example, the statistical power of our analysis was limited because of the heterogeneity of our data, with only three years of recent observations. In

such a circumstance, one anomalous year could alter results. In our case, mean January, April, and May temperatures in 2004 and 2005 were colder than most years since 1990 (Fig. 1). Thus, our estimates of changes in flowering times are probably quite conservative. In addition, we, Thoreau, and Hosmer observed flowering times throughout Concord, while Logemann observed flowering times only on her property in Concord. Because Logemann observed a smaller area and fewer plants, the first flowering dates she observed for many species were later than they were for the other observers (data not shown). Many phenological records document changes in first observations, such as first flowering dates rather than mean flowering dates. Changes in population size or sampling effort can affect these first observations independently of changes in the changes in the population mean (Tryjanowski and Sparks 2001). If populations decline over time or if sampling intensity declines, first observations can occur later even when the population mean does not change. Similarly, if populations increase over time or if sampling intensity increases, first observations can occur earlier even when the population mean does not change. Based on Hosmer's descriptions of species abundance, population sizes in Concord remained fairly constant over the last century for 32 of 43 of the main study species. Population sizes declined over time for the remaining 11 species, meaning that estimates of changes in

flowering times are probably overly conservative for these 11 species. Clearly, it is critical that researchers combine phenological data with descriptions of observation methods and changes in population sizes. Given these caveats, however, observations made by different individuals, or even using different methods, can yield surprisingly high quality, consistent results (Miller-Rushing et al. 2006).

Because of the clear ecological and evolutionary importance of phenological responses to climate change, we suggest that researchers increase efforts to collect long-term phenological data via new projects and searches of historical records. Many Long-term Ecological Research (LTER) sites, as well as other research sites, already collect such data. In addition, phenological data sets already exist in many libraries, herbaria, museums, and private homes (Ledneva et al. 2004, Primack et al. 2004, Miller-Rushing et al. 2006). Thoreau's and Hosmer's records were freely available at various libraries, but had never been previously analyzed. Logemann quite willingly shared her notebooks and charts, which she kept in her home. By using such pre-existing records and adding new sites for phenological studies, researchers could greatly enhance our understanding of how phenological changes vary according to location and species and how they might affect other aspects of ecology and evolution (Betancourt and Schwartz 2005).

In addition, evidence of phenological changes can improve public awareness of the effects that climate change is already having on biological systems. People can see changes in phenology in their immediate environment: plants flowering in gardens, fruits ripening, and birds arriving at bird feeders. We believe that building on the observations of a well known figure such as Thoreau can show that plants are responding to climate change and increase the potential for public outreach. Other studies of changes in phenology made by famous individuals such as Aldo Leopold (Bradley et al. 1999), or in well-known locations such as Washington, D.C. (Abu-Asab et al. 2001) and Boston (Primack et al. 2004, Miller-Rushing et al. 2006) generate similar public interest. Thoreau was keenly aware of the importance of educating people about environmental issues. He helped his townsmen to appreciate wild nature, and he encouraged them to protect it. He wrote, "I think that each town should have a park, or rather a primitive forest of five hundred or a thousand acres, either in one body or several, where a stick should never be cut for fuel, nor for the navy, nor to make wagons, but stand and decay for higher uses—a common possession forever, for instruction and recreation." Residents of Concord and the government have followed this advice; about 40% of Concord's land is preserved in parks and protected areas. With the help of these protected areas, we have been able to continue the same observations of flowering times made by Thoreau at the same localities in Concord. We now hope that

Thoreau's observations and our own work will promote broad discussion of the effects of climate change on biological systems. Only with an understanding of the changes taking place can people make informed decisions regarding climate change.

Literature Cited

Abu-Asab, M. S., P. M. Peterson, S. G. Shetler, and S. S. Orli. 2001. Earlier plant flowering in spring as a response to global warming in the Washington, DC, area. Biodiversity and Conservation 10:597–612.

Barbraud, C., and H. Weimerskirch. 2006. Antarctic birds breed later in response to climate change. Proceedings of the National Academy of Sciences (USA) 103:6248–6251.

Beaumont, L. J., I. A. W. McAllan, and L. Hughes. 2006. A matter of timing: changes in the first date of arrival and last date of departure of Australian migratory birds. Global Change Biology 12:1339–1354.

Betancourt, J. L., and M. D. Schwartz. 2005. Implementing a U.S. national phenology network. Eos 86:539–542.

Both, C., S. Bouwhuis, C. M. Lessells, and M. E. Visser. 2006. Climate change and population declines in a long-distance migratory bird. Nature 441:81–83.

Bradley, N. L., A. C. Leopold, J. Ross, and W. Huffaker. 1999. Phenological changes reflect climate change in Wisconsin. Proceedings of the National Academy of Sciences (USA) 96: 9701–9704.

Cayan, D. R., S. A. Kammerdiener, M. D. Dettinger, J. M. Caprio, and D. H. Peterson. 2001. Changes in the onset of spring in the western United States. Bulletin of the American Meteorological Society 82:399–415.

Chmielewski, F. M., and T. Rötzer. 2001. Response of tree phenology to climate change across Europe. Agricultural and Forest Meteorology 108:101–112.

Chuine, I. 2000. A unified model for budburst of trees. Journal of Theoretical Biology 207:337–347.

Eaton, R. J. 1974. A flora of Concord. Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts, USA.

Edwards, M., and A. J. Richardson. 2004. Impact of climate on marine pelagic phenology and trophic mismatch. Nature 430: 881–884.

Fitter, A. H., and R. S. R. Fitter. 2002. Rapid changes in flowering time in British plants. Science 296:1689–1691.

Fitter, A. H., R. S. R. Fitter, I. T. B. Harris, and M. H. Williamson. 1995. Relationships between first flowering date and temperature in the flora of a locality in central England. Functional Ecology 9:55–60.

Gordo, O., L. Brotons, X. Ferrer, and P. Comas. 2005. Do changes in climate patterns in wintering areas affect the

timing of the spring arrival of trans-Saharan migrant birds? Global Change Biology 11:12–21.

Grabherr, G., M. Gottfried, and H. Pauli. 1994. Climate effects on mountain plants. Nature 369:448.

Gross, R. S., and P. A. Werner. 1983. Relationships among flowering phenology, insect visitors, and seed-set of individuals: experimental studies on four co-occurring species of goldenrod (Solidago: Compositae). Ecological Monographs 53:95–117.

Hartmann, D. L. 1994. Global physical climatology. Academic Press, San Diego, California, USA.

Hosmer, A. W. 1878–1903. Alfred W. Hosmer Botanical Manuscripts, 1878–1903. William Munroe Special Collections. Concord Free Public Library, Concord, Massachusetts, USA.

Hosmer, A. W. 1899a. On the plants introduced by Minot Pratt at Concord, Massachusetts. Rhodora 1:170–172.

Hosmer, A. W. 1899b. Further additions to the flora of Middlesex County, Mass. Rhodora 1:223–224.

Inouye, D. W. 2008. Effects of climate change on phenology, frost damage, and floral abundance of montane wildflowers. Ecology 89:353–362.

Inouye, D. W., B. Barr, K. B. Armitage, and B. D. Inouye. 2000. Climate change is affecting altitudinal migrants and hibernating species. Proceedings of the National Academy of Sciences (USA) 97:1630–1633.

Inouye, D. W., F. Saavedra, and W. Lee-Yang. 2003. Environmental influences on the phenology and abundance of flowering by Androsace septentrionalis (Primulaceae). American Journal of Botany 90:905–910.

Kudo, G., T. Y. Ida, and T. Tani. 2008. Linkages between phenology, pollination, photosynthesis, and reproduction in deciduous forest understory plants. Ecology 89:321–331.

Ledneva, A., A. J. Miller-Rushing, R. B. Primack, and C. Imbres. 2004. Climate change as reflected in a naturalist's diary, Middleborough, Massachusetts. Wilson Bulletin 116:224–231.

McLaughlin, J. F., J. J. Hellmann, C. L. Boggs, and P. R. Ehrlich. 2002. Climate change hastens population extinctions. Proceedings of the National Academy of Sciences (USA) 99:6070–6074.

Menzel, A., and P. Fabian. 1999. Growing season extended in Europe. Nature 397:659.

Miller-Rushing, A. J., and R. B. Primack. In press. A comparison of the impacts of winter temperatures on two birch (Betula) species. Tree Physiology.

Miller-Rushing, A. J., R. B. Primack, D. Primack, and S. Mukunda. 2006. Photographs and herbarium specimens as tools to document phenological changes in response to global warming. American Journal of Botany 93:1667–1674.

New England Regional Assessment Group. 2001. New England regional assessment. University of New Hampshire, Institute for the Study of Earth, Oceans, and Space, Durham, New Hampshire, USA.

Ollerton, J., and A. Lack. 1998. Relationships between flowering phenology, plant size and reproductive success in Lotus corniculatus (Fabaceae). Plant Ecology 139:35–47.

Parmesan, C., et al. 1999. Poleward shifts in geographical ranges of butterfly species associated with regional warming. Nature 399:579–583.

Parmesan, C., and G. Yohe. 2003. A globally coherent fingerprint of climate change impacts across natural systems. Nature 421:37–42.

Parra-Tabla, V., and C. F. Vargas. 2004. Phenology and phenotypic natural selection on the flowering time of a deceit-pollinated tropical orchid, Myrmecophila christinae. Annals of Botany 94:243–250.

Post, E. S., C. Pedersen, C. C. Wilmers, and M. C. Forchhammer. 2008. Phenological sequences reveal aggregate life history response to climatic warming. Ecology 89:363–370.

Post, E., and N. C. Stenseth. 1999. Climatic variability, plant phenology, and northern ungulates. Ecology 80:1322–1339.

Pounds, J. A., et al. 2006. Widespread amphibian extinctions from epidemic disease driven by global warming. Nature 439:161–167.

Primack, D., C. Imbres, R. B. Primack, A. J. Miller-Rushing, and P. Del Tredici. 2004. Herbarium specimens demonstrate earlier flowering times in response to warming in Boston. American Journal of Botany 91:1260–1264.

Rich, P. M., D. D. Breshears, and A. B. White. 2008. Phenology of mixed woody–herbaceous ecosystems following extreme events: net and differential responses. Ecology 89:342–352.

Root, T. L., J. T. Price, K. R. Hall, S. H. Schneider, C. Rosenzweig, and J. A. Pounds. 2003. Fingerprints of global warming on wild animals and plants. Nature 421:57–60.

Schwartz, M. D., and X. Chen. 2002. Examining the onset of spring in China. Climate Research 21:157–164.

Schwartz, M. D., and B. E. Reiter. 2000. Changes in North American spring. International Journal of Climatology 20:929–932.

Seager, R., D. S. Battisti, J. Yin, N. Gordon, N. Naik, A. C. Clement, and M. A. Cane. 2002. Is the Gulf Stream responsible for Europe's mild winters? Quarterly Journal of the Royal Meteorological Society 128:2563–2586.

Shaver, G. R., J. Canadell, F. S. Chapin, J. Gurevitch, J. Harte, G. Henry, P. Ineson, S. Jonasson, J. Mellio, L. Pitelka, and L. Rustad. 2000. Global warming and terrestrial ecosystems: a conceptual framework for analysis. BioScience 50:871–882.

Sparks, T. H., and P. D. Carey. 1995. The responses of species to climate over two centuries: an analysis of the Marsham phenological record, 1736–1947. Journal of Ecology 83:321–329.

Sparks, T. H., E. P. Jeffree, and C. E. Jeffree. 2000. An examination of the relationship between flowering times and temperature at the national scale using long-term phenological records from the UK. International Journal of Biometeorology 44:82–87.

Stenseth, N. C., and A. Mysterud. 2002. Climate, changing phenology, and other life history traits: nonlinearity and match-mismatch to the environment. Proceedings of the National Academy of Sciences (USA) 99:13379–13381.

Sung, S., and R. M. Amasino. 2004. Vernalization and epigenetics: how plants remember winter. Current Opinion in Plant Biology 7:4–10.

Thoreau, H. D. 1962. The journal of Henry D. Thoreau. B. Torrey and F. H. Allen, editors. Dover Publications, New York, New York, USA.

Thoreau, H. D. 1993. Faith in a seed: the dispersion of seeds and other late natural history writings. B. P. Dean, editor. Island Press, Washington, D.C., USA.

Thoreau, H. D. 1999. Wild fruits: Thoreau's rediscovered last manuscript. B. P. Dean, editor. W. W. Norton and Company, New York, New York, USA.

Thórhallsdóttir, T. E. 1998. Flowering phenology in the central highland of Iceland and implications for climatic warming in the Arctic. Oecologia 114:43–49.

Tryjanowski, P., and T. H. Sparks. 2001. Is the detection of the first arrival date of migrating birds influenced by population size? A case study of the Red-backed Shrike Lanius collurio. International Journal of Biometeorology 45:217–219.

Visser, M. E., and C. Both. 2005. Shifts in phenology due to global climate change: the need for a yardstick. Proceedings of the Royal Society of London, B 272:2561–2569.

Walther, G. R., E. Post, P. Convey, A. Menzel, C. Parmesan, T. J. C. Beebee, J. M. Fromentin, O. Hoegh-Guldberg, and F. Bairlein. 2002. Ecological responses to recent climate change. Nature 416:389–395.

SPECIAL FEATURE

Herbarium Specimens Demonstrate Earlier Flowering Times in Response to Warming in Boston[1]

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Museum specimens collected in the past may be a valuable source of information on the response of species to climate change. This idea was tested by comparing the flowering times during the year 2003 of 229 living plants growing at the Arnold Arboretum in Boston, Massachusetts, USA, with 372 records of flowering times from 1885 to 2002 using herbarium specimens of the same individual plants. During this period, Boston experienced a 1.5°C increase in mean annual temperature. Flowering times became progressively earlier; plants flowered 8 d earlier from 1980 to 2002 than they did from 1900 to 1920. Most of this shift toward earlier flowering times is explained by the influence of temperature, especially temperatures in the months of February, March, April, and May, on flowering time. Plants with a long flowering duration appear to be as useful for detecting responses to changing temperatures as plants with a short flowering duration. Additional studies using herbarium specimens to detect responses to climate change could examine specimens from specific, intensively collected localities, such as mountain peaks, islands, and unique habitats.

Key words: Arnold Arboretum; climate change; flowering times; herbarium specimens; phenology; temperature.

Phenological observations provide one of the best biological indicators of climate change (Schwartz, 1999; Peñuelas and Filella, 2001). A growing number of papers have demonstrated phenological responses, such as earlier dates for flowering and bird migration, to changes in temperature at specific localities (e.g., Inouye and McGuire, 1991; Oglesby and Smith, 1995; Sparks and Carey, 1995; Ahas, 1999; Bradley et al., 1999; Fitter and Fitter, 2002). These phenological changes have been shown to impact interspecific interactions and evolutionary processes (Harrington et al., 1999; Inouye et al., 2000; Bradshaw and Holzapfel, 2001; Visser and Holleman, 2001). Phenological responses to climate change have been shown to exist at the global scale (Myneni et al., 1997; Walther et al., 2002; Parmesan and Yohe, 2003; Root et al., 2003). Each of these studies relies upon long-term data sets typically created for the specific purpose of measuring phenology. Unfortunately, these data sets are rare and often difficult to find, and long-term phenological data are not available for many regions and species.

To supplement this small number of specialized historical records, biological collections from museums, herbaria, zoos, botanical gardens, and research stations may provide data for examining patterns of response to changing climate. Data from such collections has many advantages over the more conventionally used historical data sets:

1. Although most current long-term phenological studies are confined to Europe and North America, herbarium and museum samples have been collected from locations across the globe, and zoos and botanical gardens are similarly dispersed.

2. Collection records at herbaria and museums often extend back more than 100 yr, providing information for a significantly longer period than do most long-term data sets that have been analyzed.

3. Many of the recent analyses of historical phenology records have been limited by data sets that include a relatively small number of species. However, the records of museums and herbaria often include samples from a large number of species.

4. Historical data sets often describe only the beginning of reproduction for an entire population, the timing of which could be altered by changes in population size as well as climate (Sparks, 1999; Tryjanowski and Sparks, 2001). On the other hand, many specimens are collected at the peak of reproduction—especially plants, which are often preserved at the flowering stage—a time that is resistant to changes in population size.

5. Plants grown in controlled conditions may experience reduced fluctuations in nutrient availability, competition, and herbivory, factors which may impact a species' response to climate change. Thus, specimens taken from botanical gardens might better show the impacts of various climatic variables on phenology, while controlling for other factors.

If records from these collections could be used to detect patterns of species response to climate change, we would have a greatly expanded range of data for research.

The purpose of this project was to test whether herbarium records could be used to detect long-term changes in flowering times and the responses of numerous species to changes in springtime temperature. As far as we know, this is the first attempt to use museum specimens for this purpose. To accomplish this, we compared the current flowering dates of marked individuals with their past flowering dates using herbarium specimens collected over the last century at the Arnold Arboretum in Boston, Massachusetts, USA.
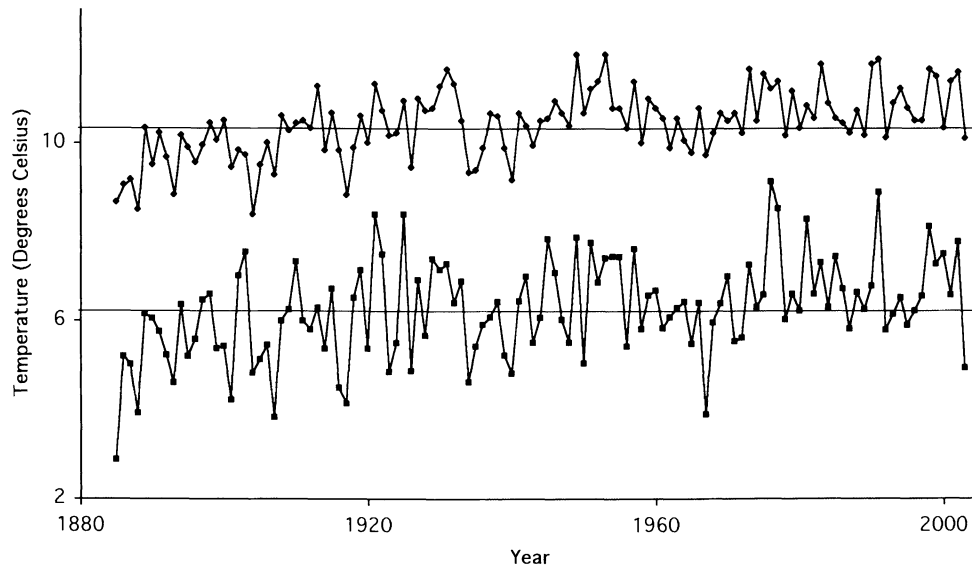
Fig. 1.   Boston temperatures from 1885 to 2003 as reported by National Oceanic and Atmospheric Administration (2004). The top series (diamonds) represents mean annual temperatures. The bottom series (squares) represents mean temperatures in February, March, April, and May. The two horizontal lines represent the long-term mean temperatures for each series (annual = 10.3°C; Feb–May = 6.1°C).

MATERIALS AND METHODS

The Arnold Arboretum, managed by Harvard University, is the oldest arboretum in the United States. It has a collection of 15 000 living woody plants and an associated herbarium of 80 000 specimens, many of which were taken from numbered plants still growing on the grounds. Herbarium specimens are dried, flattened plant specimens, mounted on sheets, with label information describing when and where they were collected. Often plants are collected in full flower for use in later studies of plant taxonomy and morphology. After examining these herbarium specimens and using our knowledge of species biology, living plants were selected for study based on the following criteria: (1) plants that produce conspicuous, easily recognizable flowers; (2) plants that have an abrupt onset and fairly rapid decline of flowers, i.e., bloom for a relatively short time; (3) plants that represent wild species (either native or introduced) rather than cultivars and hybrids, to minimize unknown alterations of plant physiology; and of greatest importance, (4) only individuals for which there was at least one herbarium record of that plant in peak flower (at least half of the flowers were open) were selected for this study. Using these criteria, we selected 229 living plants for which there were 372 herbarium records of time of flowering between 1885 and 2002 (see Supplemental Data accompanying the online version of this article); some individual plants were represented by more than one herbarium specimen. These plants were contained in 37 genera. Genera that had at least 10 individuals in the sample are *Amelanchier, Cornus, Corylopsis, Enkianthus, Halesia, Magnolia, Malus, Prunus, Rhododendron,* and *Syringa.* All specimens are woody plants, including trees, shrubs, and vines. Individual plants are grown well spaced in conditions considered ideal for the species, which includes mulching, weeding, and pesticide and fertilizer applications when needed.

During the spring and summer of 2003, the same two people observed these individually numbered plants weekly between 13 April and 14 July. The observers determined the current peak flowering date and duration of flowering for each plant. Plants were recorded as being in one of four stages: not flowering, almost in full flower, full flower, or past full flower. A plant in full flower was defined as having at least 50% of its buds in full bloom and as being suitable for making a herbarium specimen. Once a plant was recorded as past flower, it was no longer observed.

A single Julian date of full flower was determined for each plant in 2003, although this date could have missed the true flowering peak by 3–4 d due to sampling just once a week. In cases when full flowering was observed on multiple dates, the mean of the Julian dates for those days was used. Once the date of full flowering was determined for each plant in 2003, these dates

were compared with flowering dates based on the herbarium records. For each record, the Julian date of peak flowering in 2003 was subtracted from the Julian date of the past flowering date to estimate a change in plant flowering dates. In effect, the flowering dates of 2003 were used as a standard against which flowering times in other years were compared. The spring (February through May) of 2003 was colder than any previous year since 1967 and was more typical of temperatures early in the 20th century. Using these changes in flowering dates for individual plants, we used multiple regression analysis to examine how flowering times across all species have changed over time and how this change compares to the trend of warming spring temperatures in Boston. We estimated the following equation: $\Delta FT = \acute{\alpha} + B_1\Delta Temp + B_2\Delta Time + \mu$, where ΔFT, $\Delta Temp$, and $\Delta Time$ are the difference between the flowering time, temperature, and years, respectively, in 2003 and a past year in which a herbarium specimen was collected. $\acute{\alpha}$ is a constant, B_1 and B_2 are regression coefficients, and μ is a normally distributed random error term.

Over the last 100 yr, Boston has experienced an annual temperature increase of 1.5°C (Fig. 1), which has been due to regional climate change and the urban heat island effect (New England Regional Assessment, 2001). We hypothesized that, given this warming trend, analysis of herbarium samples would demonstrate that plants are responding to a warmer climate by flowering earlier. We believed that the main drawback of using herbarium samples to determine peak flowering date would be the deviation between the dates of collection and peak flowering; that is, people in the past might have collected specimens early or late in the flowering season, obscuring trends in flowering times. We investigated this area further in our analysis.

RESULTS

Over the last 100 yr (without considering temperature as an explanatory variable), plants are flowering progressively earlier, about 8 d earlier on average (Fig. 2). As seen in Fig. 2, the flowering times of plants from 1900 to 1920 are indistinguishable from their flowering times in the cool year of 2003, while plants flowering during the warmer years of 1980 to 2002 flowered much earlier than they did in the 2003 benchmark year.

We were concerned that three factors—outlying data points, non-normal distribution of collection effort, and errors associated with collection times of herbarium specimens—could
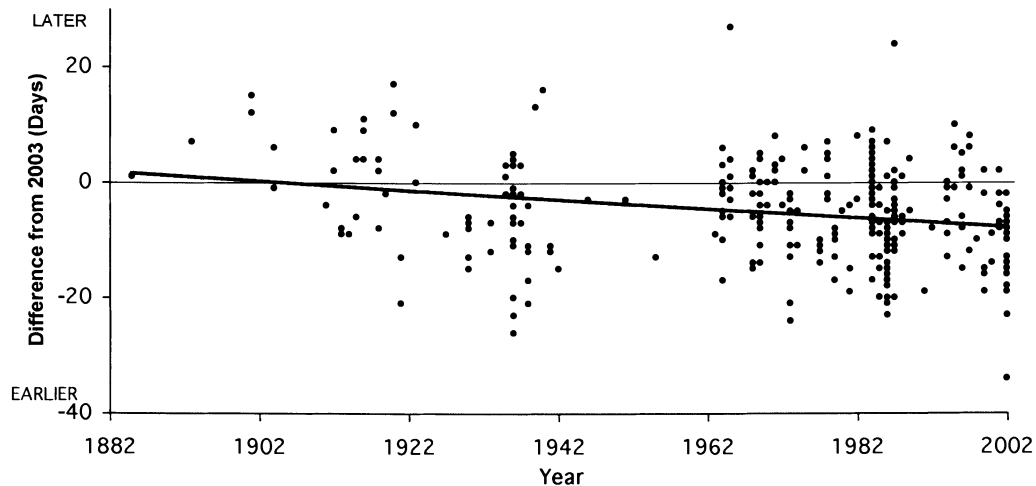
Fig. 2. Changes in flowering times of plants at the Arnold Arboretum over time: number of days plants flowered earlier or later in the past than they did in 2003 calculated as the Julian date the herbarium specimen was collected subtracted from the peak flowering date in 2003. Negative values indicate that a plant flowered on an earlier date than that it did in 2003. The line is the best fit line for the series.

have obscured or skewed this trend. Because of the large number of data points (372 samples), the few outliers present in the data set—such as a dogwood (*Cornus mas*) that flowered 27 d later in 1965 than in 2003 and a cherry tree (*Prunus apetela*) that flowered 24 d later in 1987 than in 2003—did not significantly affect the trend toward earlier flowering. Additionally, although herbarium samples were collected more actively in some decades than others—with a gap in collecting from 1940 to 1960—the overall tendency toward earlier flowering time in recent years was not affected. When the groups of herbarium samples on either side of the gap in the record are analyzed separately, observations in both periods demonstrate significant trends toward earlier flowering (1885–1955, $P < 0.001$; 1960–2002, $P < 0.001$).

In addition to the trend toward earlier flowering over time, the herbarium records demonstrate that plant flowering times are highly responsive to changes in average temperatures in the 4 mo (mean temperature in February, March, April, and

May) before and during flowering ($P < 0.001$; Fig. 3). Flowering times are sensitive to relatively small shifts in temperature, advancing 3.9 d per 1°C increase in mean spring temperature (when controlling for time). This rate of advancement agrees with the findings of other studies, which have observed flowering times to be 2–10 d earlier per 1°C increase in temperature (Fitter et al., 1995; Sparks and Carey, 1995; Sparks et al., 2000; Cayan et al., 2001). Given that temperatures in February through May have warmed approximately 1.5°C over the past 100 yr (Fig. 1), warming temperatures seem to have caused the Arboretum plants to flower approximately 5 d earlier over the past 100 yr. The multiple regression results also showed that time (after controlling for changes in temperature) showed a significant relationship with flowering time, with plants flowering earlier over time ($P < 0.001$).

We examined possible sampling errors associated with herbarium specimens. We wanted to determine if past herbarium dates for plants with a long flowering duration in 2003 would
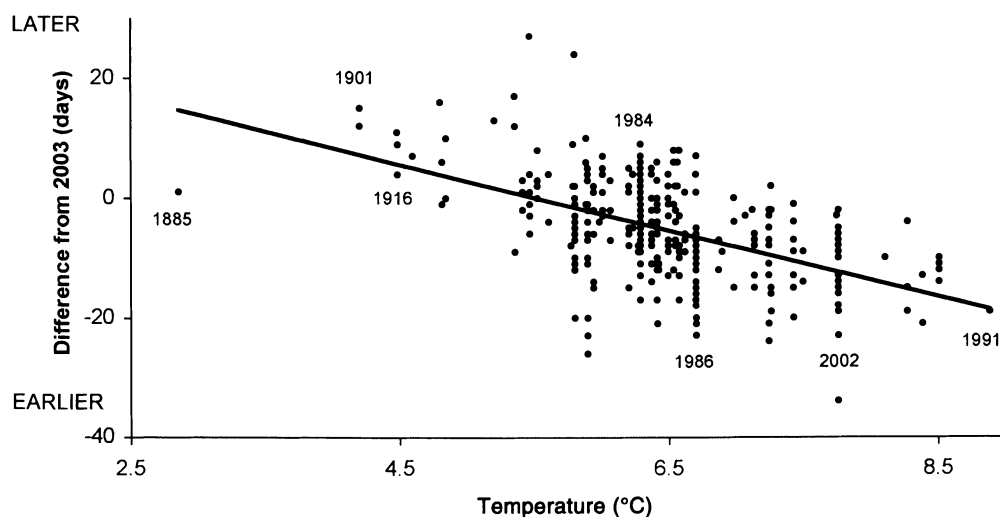

Fig. 3. Changes in flowering times of plants at the Arnold Arboretum as temperatures increase: number of days plants flowered earlier or later in the past than they did in 2003 in relation to the average temperatures in the February, March, April and May preceding flowering. Years are indicated for certain years with many specimens and years with extreme temperatures. The line is the best fit line for the series.

deviate more from the 2003 peak flowering date than would those for plants with a short flowering duration in 2003. Our hypothesis was that collectors would have a period of several weeks to collect specimens from long-flowering plants, leading to more sampling variation than plants that flower for a brief duration. To test this hypothesis, we divided plants into three categories based on their flowering in 2003: rapid flowering plants (174 records) with observed peak flowering of 1 wk or less; medium-flowering plants (115 records) with 2 wk of peak flowering; and long-flowering plants (83 records) with 3 or more weeks of peak flowering. We then examined the absolute mean difference of each plant's herbarium collection date from its date of peak flowering in 2003. We calculated the mean differences for each of the three categories of plants. We found that the mean differences were essentially the same for each category of flowering duration (means and standard deviations for rapid-, medium-, and long-flowering durations are 8.2 ± 6.2, 8.0 ± 5.8, and 7.6 ± 5.7, respectively). Therefore, we concluded that, for the purposes of this study, collection bias did not affect data from plants with a long flowering duration.

DISCUSSION

Using the herbarium records of the Arnold Arboretum from 1885 to 2002 and observations from one field season in 2003, we were able to demonstrate a significant response of plant flowering time to changing spring temperatures over the past century. Specifically, plants are now flowering earlier because of warmer spring temperatures, as shown by multiple regression.

We believe that four primary factors contributed to our ability to show this response. First, the large number of samples used from the Arboretum herbarium (372 specimens) appears to have overcome any possible error introduced by collection dates that vary from peak flowering date. Second, the samples come from one relatively homogenous location—that is, the Arboretum grounds contain no significant shifts in elevation, and land use has remained the same. These characteristics minimized sampling errors that might have hidden the effects of climate change. Third, we were able to compare past flowering times from herbarium samples with the current flowering time of the same plants that are still living on the Arboretum grounds. Thus, we were able to observe the flowering phenology of each individual plant for one field season (2003). That one year of data became our reference year, to which we compared the historical flowering times and from which we were able to establish trends in flowering date over time and temperature. By using tagged plants, we were able to eliminate the variation in flowering time among plants of the same species caused by genetic and environmental variation.

Fourth, our study benefited from the excess warming in Boston caused by the urban heat-island effect. Between 1885 and the present, the time covered by our study, the mean annual temperature of the rural areas of Massachusetts warmed by 0.7°C (Keim et al., 2003), while the city of Boston warmed by 1.5°C, as the city surfaces were covered by more buildings and paved surfaces. The extra warming almost certainly made the trend toward earlier flowering time in Boston more visible than it would have been in other, less urbanized areas of the United States (Roetzer et al., 2000). Such earlier flowering has similarly been noted in other urban centers, such as the Washington, D.C., area (Shetler and Wiser, 1987). However, the

large sample size used in our study would have likely allowed us to detect earlier flowering with even less warming than the 1.5°C warming experienced by Boston.

When we used multiple regression to control for temperature, plants were still flowering earlier over time. Therefore, factors other than temperature at the Boston weather station were also affecting flowering times. These factors could include temperature in other months of the year and other climatic variables, such as rainfall and humidity. Local conditions within and around the Arboretum may also affect flowering times. For example, increased paving of roads within the Arboretum and construction of buildings on adjacent land may have caused localized warming. Finally, if plants were flowering over a longer period as they increased in size and age and were consistently collected at the beginning of their flowering period, there could be a false trend toward earlier flowering over time. Further investigations are needed in order to determine the relative importance of these factors.

Our results suggest that other museum and herbarium collections could be utilized to measure the effects of climate change on phenological events. We believe that many such intensive collections exist at other institutions. Collections may also exist in a much more dispersed form, with samples having been collected from one location by many individuals and now being held at various storage sites. Certain localities with unusual concentrations of endemic or rare species have been intensively collected by biologists at many periods in the past, especially mountain peaks, islands, swamps, lake shores, and dunes. For example, biologists have collected extensively from many isolated natural areas—e.g., the top of Mount Washington in New Hampshire, the Florida Everglades, the northern tip of Newfoundland, and Stewart Island off the southern coast of New Zealand.

If information on flowering time from one of these locations could be gathered into one data set, an analysis could reflect the responses of native species to climate change. We believe that many such data sets from around the world could be assembled, covering the last 100–150 yr. Using such data, analyses could allow scientists to clarify the extent and character of local variation in natural responses to climate change. Furthermore, this would improve predictions of the effects that future climate change might have on biological communities. Using herbarium specimens from the Arnold Arboretum and 1 yr of observation, we have been able to demonstrate a clear pattern of earlier flowering over time and earlier flowering in response to warmer spring temperatures.

LITERATURE CITED

AHAS, R. 1999. Long-term phyto-, ornitho- and ichthyophenological time-series analyses in Estonia. *International Journal of Biometeorology* 42: 119–123.

BRADLEY, N. L., A. C. LEOPOLD, J. ROSS, AND W. HUFFAKER. 1999. Phenological changes reflect climate change in Wisconsin. *Proceedings of the National Academy of Sciences, USA* 96: 9701–9704.

BRADSHAW, W. E., AND C. M. HOLZAPFEL. 2001. Genetic shift in photoperiodic response correlated with global warming. *Proceedings of the National Academy of Sciences, USA* 98: 14509–14511.

CAYAN, D. R., S. A. KAMMERDIENER, M. D. DETTINGER, J. M. CAPRIO, AND D. H. PETERSON. 2001. Changes in the onset of spring in the western United States. *Bulletin of the American Meteorological Society* 82: 399–415.

FITTER, A. H., AND R. S. R. FITTER. 2002. Rapid changes in flowering time in British plants. *Science* 296: 1689–1691.

FITTER, A. H., R. S. R. FITTER, I. T. B. HARRIS, AND M. H. WILLIAMSON.

1995. Relationships between first flowering date and temperature in the flora of a locality in central England. *Functional Ecology* 9: 55–60.

HARRINGTON, R., I. WOIWOD, AND T. SPARKS. 1999. Climate change and trophic interactions. *Trends in Ecology and Evolution* 14: 146–150.

INOUYE, D. W., B. BARR, K. B. ARMITAGE, AND B. D. INOUYE. 2000. Climate change is affecting altitudinal migrants and hibernating species. *Proceedings of the National Academy of Sciences, USA* 97: 1630–1633.

INOUYE, D. W., AND A. D. MCGUIRE. 1991. Effects of snowpack on timing and abundance of flowering in *Delphinium nelsonii* (Ranunculaceae): implications for climate change. *American Journal of Botany* 78: 997–1001.

KEIM, B. D., A. M. WILSON, C. P. WAKE, AND T. G. HUNTINGTON. 2003. Are there spurious temperature trends in the United States Climate Division database? *Geophysical Research Letters* 30: 1404.

MYNENI, R. B., C. D. KEELING, C. J. TUCKER, G. ASRAR, AND R. R. NEMANI. 1997. Increased plant growth in the northern latitudes from 1981 to 1991. *Nature* 386: 698–702.

NATIONAL OCEANIC AND ATMOSPHERIC ADMINISTRATION. 2004. http://www. erh.noaa.gov/er/box/climate/bosave.shtml and http://www.erh.noaa.gov/box/dailystns.shtml

NEW ENGLAND REGIONAL ASSESSMENT. 2001. Preparing for a changing climate: the potential consequences of climate variability and change. The New England Regional Assessment Overview. The U.S. Global Change Program, University of New Hampshire, Durham, New Hampshire, USA.

OGLESBY, R. T., AND T. R. SMITH. 1995. Climate change in the Northeast. *In* Our living resources. U.S. Department of the Interior National Biological Service, Washington, D.C., USA.

PARMESAN, C., AND G. YOHE. 2003. A globally coherent fingerprint of climate change impacts across natural systems. *Nature* 421: 37–42.

PEÑUELAS, J., AND I. FILELLA. 2001. Responses to a warming world. *Science* 294: 793–794.

ROETZER, T., M. WITTENZELLER, H. HAECKEL, AND J. NEKOVAR. 2000. Phenology in central Europe—differences and trends of spring phenophases in urban and rural areas. *International Journal of Biometeorology* 44: 60–66.

ROOT, T. L., J. T. PRICE, K. R. HALL, S. H. SCHNEIDER, C. ROSENZWEIG, AND J. A. POUNDS. 2003. Fingerprints of global warming on wild animals and plants. *Nature* 421: 57–60.

SCHWARTZ, M. D. 1999. Advancing to full bloom: planning phenological research for the 21st century. *International Journal of Biometeorology* 42: 113–118.

SHETLER, S. G., AND S. K. WISER. 1987. First flowering dates for spring-blooming plants of the Washington, D. C., area for the years 1970 to 1983. *Proceedings of the Biological Society of Washington* 100: 998–1017.

SPARKS, T. H. 1999. Phenology and the changing pattern of bird migration in Britain. *International Journal of Biometeorology* 42: 134–138.

SPARKS, T. H., AND P. D. CAREY. 1995. The responses of species to climate over two centuries: an analysis of the Marsham phenological record, 1736–1947. *Journal of Ecology* 83: 321–329.

SPARKS, T. H., E. P. JEFFREE, AND C. E. JEFFREE. 2000. An examination of the relationship between flowering times and temperature at the national scale using long-term phenological records from the UK. *International Journal of Biometeorology* 44: 82–87.

TRYJANOWSKI, P., AND T. H. SPARKS. 2001. Is the detection of the first arrival date of migrating birds influenced by population size? A case study of the red-backed shrike *Lanius collurio*. *International Journal of Biometeorology* 45: 217–219.

VISSER, M. E., AND L. J. M. HOLLEMAN. 2001. Warmer springs disrupt the synchrony of oak and winter moth phenology. *Proceedings of the Royal Society of London Series B—Biological Sciences* 268: 289–294.

WALTHER, G. R., E. POST, P. CONVEY, A. MENZEL, C. PARMESAN, T. J. C. BEEBEE, J. M. FROMENTIN, O. HOEGH-GULDBERG, AND F. BAIRLEIN. 2002. Ecological responses to recent climate change. *Nature* 416: 389–395.

Validation of biological collections as a source of phenological data for use in climate change studies: a case study with the orchid *Ophrys sphegodes*

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Summary

1. The scarcity of reliable long-term phenological data has severely hindered the study of the responses of species to climate change. Biological collections in herbaria and museums are potential sources of long-term data for such study, but their use for this purpose needs independent validation. Here we report a rigorous test of the validity of using herbarium specimens for phenological studies, by comparing relationships between climate and time of peak flowering derived from herbarium records and from direct field-based observations, for the terrestrial orchid *Ophrys sphegodes*.
2. We examined herbarium specimens of *O. sphegodes* collected between 1848 and 1958, and recorded peak flowering time directly in one population of *O. sphegodes* between 1975 and 2006. The response of flowering time to variation in mean spring temperature (March–May) was virtually identical in both sets of data, even though they covered different periods of time which differ in extent of anthropogenic temperature change. In both cases flowering was advanced by *c.* 6 days per °C rise in average spring temperature.
3. The proportion of variation in flowering time explained by spring temperature was lower in the herbarium record than in direct field observations. It is likely that some of the additional variation was due to geographical variation in collection site, as flowering was significantly earlier at more westerly sites, which have had warmer springs, over their range of 3.44° of longitude.
4. Predictions of peak flowering time based on the herbarium data corresponded closely with observed peak flowering times in the field, indicating that flowering response to temperature had not altered between the two separate periods over which the herbarium and field data were collected.
5. *Synthesis.* These results provide the first direct validation of the use of herbarium collections to examine the relationships between phenology and climate when field-based observational data are not available.

Key-words: biological collections, climate change, flowering time, herbarium specimens, natural history collections, *Ophrys sphegodes,* Orchidaceae, phenology, spring, temperature

Introduction

Phenological events respond directly to climate. Recent climate change has undoubtedly affected the timing of development and seasonal events in many groups of organisms, including amphibians (Beebee 1995), birds (Crick *et al.* 1997), fungi (Kauserud *et al.* 2008) and plants (Sparks & Carey 1995; Fitter & Fitter 2002). Understanding the effects of recent climate change is a vital step towards predicting the consequences of future change. Moreover, only by elucidating the responses of individual species will we be able to predict the potentially disruptive effects of accelerating climate change on species interactions.

Detecting phenological trends in relation to long-term climate change is not straightforward. Because trends can be concealed by short-term inter-annual climate variation

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(Badeck *et al.* 2004), long datasets are needed. For most species, data collected specifically for the study of climate-induced phenological change are not available, or are difficult to find, reflecting the scarcity of long-term monitoring schemes (Sparks & Carey 1995). The choice of species for long-term studies of phenology has thus been dictated up to now by the availability of suitable field records. A further major obstacle is that most long-term data only record the beginning of phenological events in populations, such as dates of first flowering. Miller-Rushing, Inouye & Primack (2008) have shown that the use of such data to infer changes in phenology can be unreliable, and they advise that dates on which phenological stages reach their peak are preferable. However, long-term field-based records of the dates on which phenological events are at their peak are extremely rare.

Specimen-based records in biological collections are another potential source of data, verifiable in both space and time, for the study of climate-induced phenological change. Until recently, the potential of such records has been largely overlooked (Suarez & Tsutsui 2004), even though the only data available for studying phenological trends in many species are those held in natural history collections in museums or herbaria. Recent phenological studies have utilised less orthodox data sources, including historical archives (Aono & Kazui 2008), photographs (Miller-Rushing *et al.* 2006; Sparks, Huber & Croxton 2006; Crimmins & Crimmins 2008) and herbarium specimens (Primack *et al.* 2004; Bolmgren & Lönnberg 2005; Lavoie & Lachance 2006; Miller-Rushing *et al.* 2006; Bowers 2007; Kauserud *et al.* 2008; Gallagher, Hughes & Leishman 2009). Herbarium records are unique amongst these sources of information in that they capture an individual plant's phenological state at the time and location of collection, and therefore may represent a substitute for field observation. Herbarium specimens are also likely to have been collected when phenological stages such as flowering are near their peak, rather than at an early or late stage in such seasonal events. Recent studies suggest that herbarium collections may provide data that can be exploited in climate change studies, because findings have been broadly in line with trends reported in the phenological literature (Sparks 2007) and have supported the predictions of physiological models of phenological events such as flowering (Bowers 2007). Nevertheless, they depend on averaging-out the numerous possible influences and biases involved in a collection process that was not designed with the study of phenology in mind, within which the climatic signal-to-noise ratio might be low. Given the absence of long-term monitoring for most species, there is little direct evidence from which to evaluate the potential of averaged trends in events such as flowering time, derived from herbarium collections, as proxies for field data.

We report a critical comparison of independent field- and herbarium-derived data as predictors of flowering time in a species (the terrestrial orchid *Ophrys sphegodes*) for which a unique long-term phenological record of peak flowering time was available (Hutchings 2010). As the flowering time of plants that flower in early summer is generally advanced after warmer springs, we examined relationships between the flowering date

of *O. sphegodes* and climate in the 9 months prior to flowering. This corresponds with the period from the end of tuber dormancy to flowering in this species. Specific hypotheses were (i) that flowering date would be advanced by warmer springs, (ii) that the relationship between flowering date and mean spring temperature would be the same in data derived from herbarium records and annual field observations, and therefore (iii) that in a particular species for which this test is possible, herbarium records would be validated both as an effective proxy for long-term monitoring in climate change research and as a predictor of phenological responses to future climate change.

Materials and methods

STUDY SPECIES

Ophrys sphegodes (the early spider orchid) is a species of southern and central Europe, with a northern range limit that includes southern England. It is associated with ancient, species-rich grassland over calcareous soils. At present the species is rare in the UK, where it is largely confined to Dorset, West and East Sussex and Kent (Lang 1989; Harrap & Harrap 2005).

Although the length of the mycotrophic, subterranean phase of the life cycle of *O. sphegodes* is unclear, it is a short-lived species after its first appearance above ground, rarely flowering for more than three consecutive years. Few plants survive for more than 10 years after initial emergence (Hutchings 1987, 2010) and most survive for less than 3 years. In the UK, the leaves of *O. sphegodes* emerge above ground in September or October (Hutchings 1989). The flowering period is relatively short, commencing during late April or early May, and usually ending by late May (Lang 1989). In most populations in the UK inflorescences bear from one to six flowers (usually two or three), which open in succession from the bottom of the inflorescence. Pollination is followed by rapid withering of the flower. Sanger & Waite (1998) found that the number of inflorescences bearing ripening seed peaked at the end of June and that rapid dieback of the plant ensued; few plants remain above ground at the end of July. This relatively short reproductive period would be expected to conserve any climatically-induced phenological signal.

HERBARIUM DATA

We examined all 192 specimens of *O. sphegodes* held in herbaria at the Natural History Museum, London (BM, 133 specimens) and Royal Botanic Gardens, Kew (K, 59 specimens) to verify identification. All of the specimens originated from southern coastal counties of England (Dorset, Isle of Wight, Hampshire, East and West Sussex, and Kent), reflecting the limited historical distribution of *O. sphegodes* (Carey & Dines 2002). The geographical range of the sites from which specimens were collected was 3.44° (decimal) longitude and 0.76° (decimal) latitude. Specimens with incomplete data for site of collection and collection date were discarded. Because of the rarity of *O. sphegodes* in the UK, the dataset was comparatively small and therefore it was important to ensure that the records represented the peak flowering stage as closely as possible. For this reason only specimens with at least 60% of their flowers open were included in the study; normally most of the flowers are open at the same time in *O. sphegodes*. Some of the herbarium sheets consisted of multiple specimens mounted together. As the specimens in such cases had been collected by a single collector, on the same day and at the same location, they were treated as non-independent and the mean percentage

of open flowers was derived. Individuals in fruit or with senescent flowers were excluded.

We rejected 53% of the 192 specimens: 2 were damaged, 9 had unclear or illegible records of collection date, 31 were not dated, 60 were imprecisely dated (only the month or year), 3 were in seed, and 1 presented fewer than 60% of flowers open. Nine specimens were duplicates (multiple specimens) and therefore mean results were used. The final data set comprised 77 specimens providing at least one data point for each of 57 years, spanning a 111-year period from 1848 to 1958.

FIELD DATA

Records of the peak flowering time of *O. sphegodes* were made in 25 of the 32 years between 1975 and 2006 in a demographic study of a population consisting of many thousands of plants at Castle Hill National Nature Reserve, East Sussex, UK (Hutchings 2010). Peak flowering was based on assessment of the entire population to give a central tendency that would fit the flowering phenology of as many individual plants as closely as possible.

METEOROLOGICAL DATA

Mean monthly Central England Temperature (CET) records for the period 1848–2006 (Parker, Legg & Folland 1992) were obtained from the UK Meteorological Office (http://hadobs.metoffice.com/hadcet/cetml1659on.dat). This is the only complete climate record available for the years during which the herbarium records and field data were collected. However, data for Central England are strictly representative only for a roughly triangular area enclosed by Bristol, Preston and London (Parker, Legg & Folland 1992). This is to the north of the distribution range of *O. sphegodes*. Monthly mean temperatures were available from two Meteorological Office weather stations on the south coast, in locations corresponding with eastern and western centres of the distribution of *O. sphegodes*. Eastbourne, East Sussex UK, 21 km east of the Castle Hill field site, operated for the period during which the field records were collected. Monthly minimum and maximum temperature data were available for Southampton, to the west, for all but 5 of the 111 years of the collection period covered by the herbarium specimens. Data from both of these collection sites would be expected to represent the climate within the distribution range of *O. sphegodes* better than the climate records available from CET. The means of monthly minimum and maximum temperature were used for both stations. Historical temperature data were not available closer to any of the sites of collection of the individual specimens in the herbarium records.

ANALYSIS

The distribution of collection dates in the herbarium dataset for 1848–1958, expressed as number of days after 1 April, was checked for normality and presence of outliers. The peak flowering date for the Castle Hill population in the years 1975–2006 was similarly expressed as days after 1 April.

Both sets of flowering phenology data were examined for relationships with mean CET temperature data from the 9 months prior to the flowering season (i.e. the period of growth following breaking of tuber dormancy the previous summer). These data included mean monthly temperature and its averages over successive 3-month periods (September–November, December–February, and March–May). This was carried out to establish which temperature variables had the highest predictive power for flowering time in both sets of

Table 1. Comparison of correlations between flowering date and temperature for the herbarium records and the field data. Correlations are shown with mean temperatures for 3-monthly periods and individual months in the same year as flowering (January–May) or in the year previous to flowering (September–December). A negative correlation indicates that a higher mean temperature is associated with an earlier flowering date

Period of mean temperature	Herbarium data (1848–1958) $n = 77$	Field data (1975–2006) $n = 25$
Seasons:		
September–November	−0.004	−0.072
December–February	−0.065	−0.610**
March–May	−0.426**	−0.801**
Months:		
September	0.008	−0.273
October	0.108	0.226
November	−0.106	−0.171
December	0.047	−0.085
January	−0.003	−0.579**
February	−0.159	−0.549**
March	−0.396**	−0.609**
April	−0.153	−0.405*
May	−0.259*	−0.592**

**$P < 0.01$; *$P < 0.05$.

phenological data. Multiple regressions using mean temperatures for the individual months failed to produce a single model that could be applied to both of the datasets, because of collinearity between the variables, and the fact that the models included different individual months for the two data sets. However, the mean temperature for the 3 months from March to May had the highest individual correlation with peak flowering date in both sets of data in an analysis of single variables (Table 1). This was designated 'mean spring temperature' and was adopted as the single predictor variable in comparisons of the phenological responses in herbarium and field data. Models using mean spring temperature accounted for only marginally less variation than the best combinations of months in separate stepwise (forward) multiple regressions. In order to investigate whether distance from the weather station influenced the relationship, the phenological analysis was repeated using Eastbourne mean spring temperature data for the field phenological regressions and equivalent Southampton data for the herbarium phenological regressions.

Variation in flowering time among the herbarium specimens was further investigated using a regression on (decimalised) longitude of origin. This sought to identify geographical sources of variation.

The linear regression model derived from the herbarium data and CET was used to predict peak flowering dates from mean spring temperature for the years between 1975 and 2006 for which field observations were available. Regression analyses were carried out with spss 16 (SPSS Inc., Chicago, IL, USA). Slopes and intercepts of regressions were compared using Graphpad Prism 5 (Graphpad software Inc., La Jolla, CA, USA). Predicted flowering dates were compared with observed flowering dates using principal axis regression (Sokal & Rohlf 1969).

Results

Data derived from herbarium specimens over the 111-year period from 1848 until 1958, and recorded in the field between

1975 and 2006, both confirmed the importance of spring temperature in determining flowering time. We found significant individual correlations between peak flowering date and several measures of mean temperature in the CET records in the preceding months (Table 1). For herbarium material, there were significant correlations with mean temperature in March and May of the year of flowering but the highest correlation was with mean temperature over the 3 month period from March–May. Results for the field data were similar, but with significant correlations for January, February, March, April and May. The strongest correlation was again with the mean for the period March–May.

As predicted, warmer years were associated with earlier flowering. The regression of flowering date obtained from the herbarium specimens on mean March–May (spring) temperature (Fig. 1a) accounted for 18% of the variation in flowering time. A 1 °C increase in mean temperature between March and May was associated with an advance in flowering of 6.5 days. Analysis of the field data yielded strikingly similar results. Linear regression of flowering date on mean spring temperature accounted for 64% of the variation in date of flowering (Fig. 1b) and a 1 °C increase in mean spring temperature was associated with an advance in flowering of 6.7 days. The regression models derived from the herbarium data and field data were statistically indistinguishable: neither the gradi-

ents $(F_{1,98} = 0.0035, P = 0.952)$ nor the intercepts $(F_{1,99} = 0.0908, P = 0.764)$ were significantly different, indicating that the phenological response to temperature was the same during the different periods over which the two sets of data were collected.

Applying the same analysis with less geographically distant temperature data for the field and herbarium records gave significant and strikingly similar results. Spring temperature at Southampton accounted for 13% of the phenological variation in herbarium data (Fig. 2a) and Eastbourne temperature accounted for 59% of that in the field data. In both cases flowering advanced by 5.7 days per 1 °C increase in spring temperature. The two regressions were again statistically indistinguishable (gradients, $F_{1,93} = 0.00007, P = 0.993$; intercepts, $F_{1,94} = 0.854, P = 0.358$). Furthermore, the gradients of the two regressions of field data on temperature recorded at Eastbourne and CET were not significantly different $(F_{1,46} = 0.481, P = 0.491)$, and neither were the gradients of the two regressions with herbarium data using Southampton and CET temperature records $(F_{1,145} = 0.130, P = 0.719)$; this indicates that the predicted flowering responses of the plants to temperature were consistent irrespective of the temperature records used. In both of these comparisons the intercepts were significantly different (field data, $F_{1,47} = 14.6, P = 0.004$; herbarium data, $F_{1,146} = 10.3, P = 0.002$),
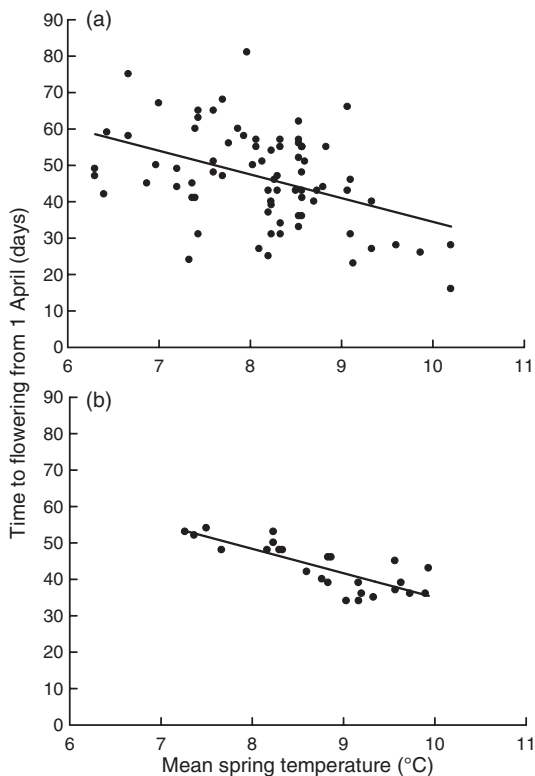

Fig. 1. Relationships between flowering date (expressed as days after 1 April) and mean spring temperature (March–May) in Central England derived from (a) herbarium records from 1848 to 1958 ($y = 99.54-6.51x$, $r^2 = 0.182$, $P < 0.001$, $n = 77$) and (b) field data between 1975 and 2006 ($y = 101.88-6.69x$, $r^2 = 0.642$, $P < 0.0001$, $n = 25$).
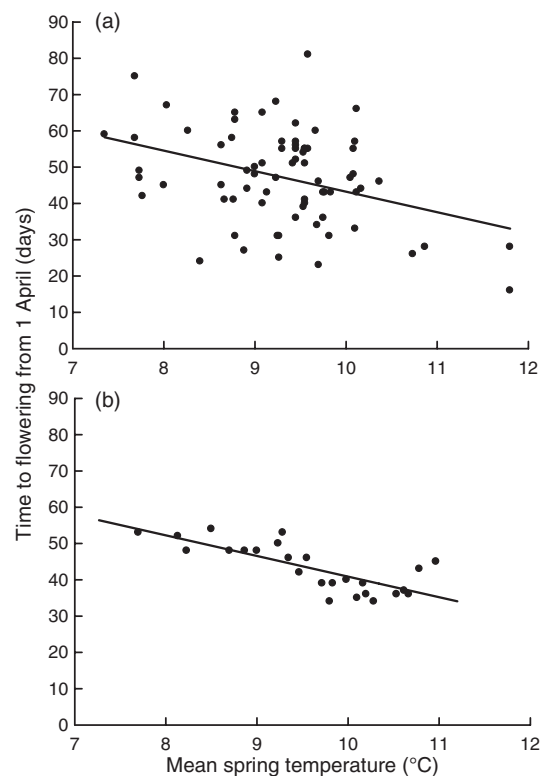
Fig. 2. Relationships between flowering date (expressed as days after 1 April) and mean spring temperature (March–May): (a) between herbarium records from 1855 to 1958 and temperature at Southampton ($y = 99.8-5.66x$, $r^2 = 0.134$, $P = 0.0016$, $n = 72$); (b) between field data from 1975 to 2006 and temperature at Eastbourne ($y = 97.7-5.68x$, $r^2 = 0.586$, $P < 0.0001$, $n = 25$).
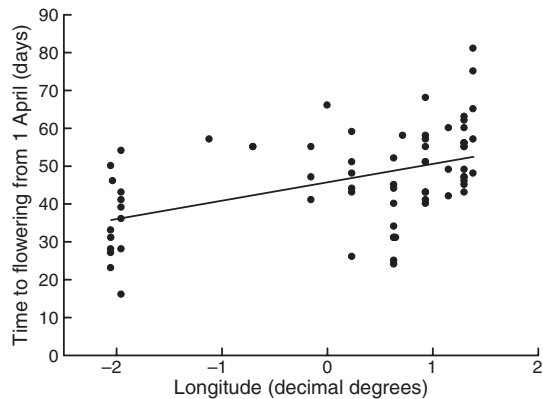
Fig. 3. Relationship between flowering date (expressed as days after 1 April) and longitude of collection site for the herbarium records. Negative values of decimalised longitude are westerly ($y = 45.74 - 4.86x$, $r^2 = 0.219$, $P = <0.001$, $n = 69$).
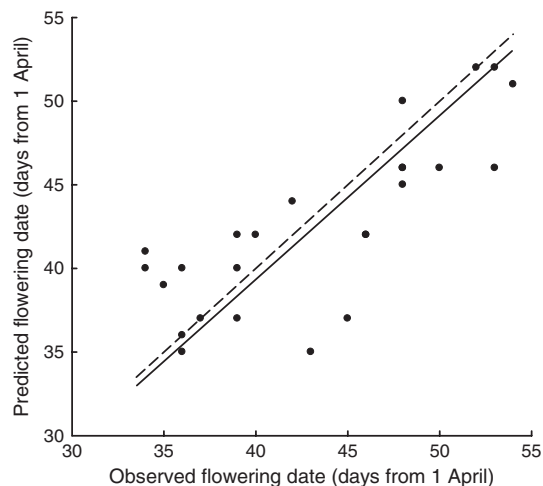

Fig. 4. Relationship between observed flowering date in the field (y_1) in 25 years between 1975 and 2006, and flowering date predicted from herbarium data for the same years (y_2). The principal axis regression (solid line) is $y_1 = -0.173 + 1.021y_2$, $r^2 = 0.63$, $P < 0.001$, $n = 25$. The dashed line would apply if there were exact correspondence between the observed flowering date and the predicted flowering date.

reflecting the differences between the temperature records used.

The effect of longitude of origin on the flowering time of herbarium specimens was significant (Fig. 3). Flowering was earlier at more westerly collection sites by an average of 4.86 days per degree longitude.

The regression model derived from herbarium specimens (1848–1958) and CET was used to predict flowering dates for each of the 25 years between 1975 and 2006 for which there were field records of time of flowering. These predictions were highly correlated with the observed peak flowering dates ($P < 0.01$); the principal axis regression between observed and predicted dates had a coefficient close to unity (1.021) and accounted for 63% of the variation (Fig. 4).

Discussion

Although biological collections can potentially provide valuable evidence of the impacts of climate change on the phenology of plant and animal species (Sparks 2007), their value as a proxy for field data has not previously been tested independently for any species. Miller-Rushing *et al.* (2006) compared flowering dates in recent benchmark years with those derived from historical photographs and herbarium specimens (1900–1921) for a range of species and found that not only were the deviations highly correlated with the corresponding differences in spring temperature but they yielded a trend that was very similar to that observed in independent field data of first flowering dates for the years 1887–1903. Bolmgren & Lönnberg (2005) established correspondence between flowering times derived from herbarium records and phenological observations, but did not investigate the underlying climatic drivers. The power of historical collection data to predict the consequences of future climate change needs to be tested directly. The availability of field data for the rare terrestrial orchid *Ophrys sphegodes*, recorded at a single site in the UK over a 32-year period, provided a unique opportunity to seek validation of the relationship between flowering date and mean spring temperature that was apparent from analysis of data from herbarium specimens collected over a much longer, and different, period of years. The comparison is greatly strengthened by the fact that peak flowering time was recorded in the field, rather than date of first flowering, which is more common in long-term phenological records. It is now clear that first flowering dates may not be ideal measures of plant responses to climate change, because the extremes of flowering distributions are more susceptible to confounding effects than central values (Miller-Rushing, Inouye & Primack 2008). Herbarium collections also tend to reflect peak flowering, as collectors generally aim to obtain prime specimens in full flower, as testified by the fact that we had to discard only one specimen in which too few flowers were open to satisfy our sampling criterion.

Both historical and contemporary data showed that the peak flowering date of *O. sphegodes* was earlier in years with warmer springs, as expected (see also Hutchings 2010). This was the case both when the two phenological records were related to a common temperature record (CET) and when field and herbarium records were related to different but more geographically proximate temperature records (Eastbourne and Southampton respectively). The close correspondence between field and herbarium regressions, irrespective of the geographical locations of the temperature records tested, argues for the robustness of the relationships. Furthermore, using geographically different temperature records did not significantly alter the results for either contemporary or historical sources of data. Previous phenological studies have found similar correlations between flowering date and measures of spring temperature in spring- and summer-flowering species. The estimated advance in peak flowering date of 5.7–6.7 days per 1 °C rise in temperature in *O. sphegodes* is within the range reported for advance in first flowering date in other species in the UK. Fitter *et al.* (1995) reported a mean advance of first flowering

date of 4.4 days per 1 °C for 243 species at a single locality but with considerable differences between species; similarly, first flowering dates of 24 species, averaged across the UK, advanced between 2 and 10 days per 1 °C increase in temperature (Sparks, Jeffree & Jeffree 2000).

The relationships between peak flowering date and spring temperature derived from contemporary and historical data for *O. sphegodes* were nearly identical, indicating a common response to spring temperature, notwithstanding that the historical collection and field observation periods were dissimilar in length, separated in time and different in geographical extent. This consistent response is important, as the pace of climate change has accelerated since 1975 when the field studies were initiated (IPCC 2007). None of the herbarium specimens was collected after 1958 and they therefore largely pre-date the period of fastest anthropogenic climate change. Because the field and herbarium data were independent, it was possible also to test the power of the earlier herbarium records to predict the effects of subsequent climatic warming. Importantly, although there was some variation between years in the accuracy of predictions, the overall predictive power was extremely good, with the principal axis regression line for predicted and observed values lying close to the ideal 1 : 1 relationship.

Rigorous validation of the type presented here, although only based on data for a single species to date, serves to increase confidence in the use of biological collections for predicting future phenological responses to climate change. Despite the strong underlying mean temperature signal, variation in flowering time may be influenced by a myriad of factors, and there are likely to be more confounding factors in the herbarium record than in the field data, because it includes specimens taken from a wider range of geographical locations and microhabitats. Predictions based solely on mean spring temperature in Central England accounted for 18% of the variation in flowering date seen in herbarium specimens, but 64% of variation in flowering date in the field records from a single site. Use of more local temperature records in fact accounted for slightly (but not significantly) less variation in both cases, possibly because of the use of minimum and maximum temperatures averaged on a monthly rather than daily basis. Another important explainable source of variation in flowering time in the herbarium record was the geographical range of collection sites, as seen in the significant regression on longitude. This was the major gradient in distribution, and earlier flowering at westerly sites is consistent with a climatic trend to warmer springs in the west. This suggests that, had local temperature records been available for each collection site, even more of the variation in flowering time would have been accounted for by spring temperature. Despite the lower signal-to-noise ratio in the herbarium record, the signal was the same as in the field data and it was applicable over a much longer period. Bowers (2007) used physiological models based on previously determined flowering requirements (trigger dates and heat sums above a 10 °C threshold) to predict, retrospectively, advancing flowering dates of shrubs in the Sonoran desert through the 20th century. A correlated tendency towards earlier collec-

tion dates in herbarium material over the same period supported the hypothesis that there had been a genuine response to changing climate, especially as there was no evidence that collector behaviour had changed over the period of study. However, the use of herbarium specimens assumes that they are representative samples of the population from which they are drawn. The potential for bias resulting from variation in collection effort has been voiced as a concern by previous authors (Case *et al.* 2007). Our study demonstrates both that collector bias is not a problem when the herbarium data accepted for use in scientific studies are subjected to carefully controlled selection criteria, and that it is not necessary to have hundreds of specimens in order to extract useful information about the relationships between climate and time of flowering. Although further validation using additional species with different phenologies is desirable, the extreme scarcity of suitable field observations limits opportunities for this to be achieved at the present time. As a spring-flowering plant, *O. sphegodes* falls into a group identified as having flowering phenologies that are likely to be particularly sensitive to temperatures early in the year (Fitter *et al.* 1995), although both the scale and direction of changes in phenology can be idiosyncratic and potentially influenced by additional climatic drivers (Crimmins, Crimmins & Bertelsen 2010). Species that flower later in the summer may be less sensitive to warmer temperatures, and species that reproduce in the autumn may be sensitive in the opposite direction; analysis of 34 500 dated herbarium records of autumn-fruiting of mushrooms in Scandinavia has revealed an average delay of 12.9 days since 1980, as the growing season has been extended by warming (Kauserud *et al.* 2008).

For most species of plants and animals, biological collections are the only source of long-term phenological data. It is estimated that some 2.5 billion specimens of flora and fauna are held in biological collections worldwide (Graham *et al.* 2004). The current drive toward digitisation of collections is facilitating the dissemination of the information they contain. An estimated 60 million records are already available for a wide range of taxa *via* internet information networks such as the Global Biodiversity Information Facility and HerpNET (Graham *et al.* 2004). With appropriate validation, the exploitation of this resource will have increasing relevance and value (Prather *et al.* 2004) as we seek to understand and predict the consequences of continuing climate change.

Acknowledgements

References

Aono, Y. & Kazui, K. (2008) Phenological data series of cherry tree flowering in Kyoto, Japan, and its application to reconstruction of springtime temperatures since the 9th century. *International Journal of Climatology*, **28**, 905–914.

Badeck, F.W., Bondeau, A., Böttcher, K., Doktor, D., Lucht, W., Schaber, J. & Sitch, S. (2004) Responses of spring phenology to climate change. *New Phytologist*, **162**, 295–309.

Beebee, T.J.C. (1995) Amphibian breeding and climate. *Nature*, **374**, 219–220.

Bolmgren, K. & Lönnberg, K. (2005) Herbarium data reveal an association between fleshy fruit type and earlier flowering time. *International Journal of Plant Science*, **166**, 663–670.

Bowers, J.E. (2007) Has climatic warming altered spring flowering date of Sonoran Desert shrubs? *The Southwestern Naturalist*, **52**, 347–355.

Carey, P.D. & Dines, T.D. (2002) Ophrys sphegodes. *New Atlas of the British Flora* (eds C.D. Preston, D.A. Pearman & T.D. Dines), p. 865. Oxford University Press, Oxford, UK.

Case, M.A., Flinn, K.M., Jancaitis, J., Alley, A. & Paxton, A. (2007) Declining abundance of American ginseng (*Panax quinquefolius* L.) documented by herbarium specimens. *Biological Conservation*, **134**, 22–30.

Crick, H.Q.P., Dudley, C., Glue, D.E. & Thomson, D.L. (1997) UK birds are laying eggs earlier. *Nature*, **388**, 526.

Crimmins, M.A. & Crimmins, T.M. (2008) Monitoring plant phenology using digital repeat photography. *Environmental Management*, **41**, 949–958.

Crimmins, T.M., Crimmins, M.A. & Bertelsen, C.D. (2010) Complex responses to climate drivers in onset of spring flowering across a semi-arid elevation gradient. *Journal of Ecology*, **98**, 1042–1051.

Fitter, A.H. & Fitter, R.S.R. (2002) Rapid changes in flowering time in British plants. *Science*, **296**, 1689–1691.

Fitter, A.H., Fitter, R.S.R., Harris, I.T.B. & Williamson, M.H. (1995) Relationships between first flowering date and temperature in the flora of a locality in central England. *Functional Ecology*, **9**, 55–60.

Gallagher, R.V., Hughes, L. & Leishman, M.R. (2009) Phenological trends among Australian alpine species: using herbarium records to identify climate-change indicators. *Australian Journal of Botany*, **57**, 1–9.

Graham, C.H., Ferrier, S., Huettman, F., Moritz, C. & Peterson, A.T. (2004) New developments in museum-based informatics and applications in biodiversity analysis. *Trends in Ecology and Evolution*, **19**, 497–503.

Harrap, A. & Harrap, S.. (2005) *Orchids of Britain and Ireland*. A & C Black Publishers Ltd, London.

Hutchings, M.J. (1987) The population biology of the early spider orchid, *Ophrys sphegodes* Mill I. A demographic study from 1975 to 1984. *Journal of Ecology*, **75**, 711–727.

Hutchings, M.J. (1989) Population biology and conservation of *Ophrys sphegodes*. *Modern Methods in Orchid Conservation: The Role of Physiology, Ecology and Management* (ed. H.W. Pritchard), pp. 101–115. Cambridge University Press, Cambridge, UK.

Hutchings, M.J. (2010) The population biology of the early spider orchid, *Ophrys sphegodes* Mill III. Demography across three decades. *Journal of Ecology*, **98**, 867–878.

IPCC (2007) *Intergovernmental Panel on Climate Change: Climate Change 2007: Impacts, Adaptation and Vulnerability*. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK.

Kauserud, H., Stige, L.C., Vik, J.O., Økland, R.H., Høiland, K. & Stenseth, N.C. (2008) Mushroom fruiting and climate change. *Proceedings of the National Academy of Sciences*, **105**, 3811–3814.

Lang, D.. (1989) *A Guide to the Wild Orchids of Great Britain and Ireland*. Oxford University Press, Oxford, UK.

Lavoie, C. & Lachance, D. (2006) A new herbarium-based method for reconstructing the phenology of plant species across large areas. *American Journal of Botany*, **93**, 512–516.

Miller-Rushing, A.J., Inouye, D.W. & Primack, R.B. (2008) How well do first flowering dates measure plant responses to climate change? The effects of population size and sampling frequency *Journal of Ecology*, **96**, 1289–1296.

Miller-Rushing, A.J., Primack, R.B., Primack, D. & Mukunda, S. (2006) Photographs and herbarium specimens as tools to document phenological changes in response to global warming. *American Journal of Botany*, **93**, 1667–1674.

Parker, D.E., Legg, T.P. & Folland, C.K. (1992) A new daily central England temperature series, 1772-1991. *International Journal of Climatology*, **12**, 317–342.

Prather, L.A., Alvarez-Fuentes, O., Mayfield, M.H. & Ferguson, C.J. (2004) The decline of plant collecting in the United States: a threat to the infrastructure of biodiversity studies. *Systematic Botany*, **29**, 15–28.

Primack, D., Imbres, C., Primack, R.B., Miller-Rushing, A.J. & Del Tredici, P. (2004) Herbarium specimens demonstrate earlier flowering times in response to warming in Boston. *American Journal of Botany*, **91**, 1260–1264.

Sanger, N.P. & Waite, S. (1998) The phenology of *Ophrys sphegodes* (the early spider orchid): what annual censuses can miss. *Botanical Journal of the Linnean Society*, **126**, 75–81.

Sokal, R.R. & Rohlf, F.J. (1969) *Biometry*. W.H.Freeman, New York, USA.

Sparks, T.H. (2007) Lateral thinking on data to identify climate impacts. *Trends in Ecology and Evolution*, **22**, 169–171.

Sparks, T.H. & Carey, P.D. (1995) The responses of species to climate over two centuries: an analysis of the Marsham phenological record 1736-1947. *Journal of Ecology*, **83**, 321–329.

Sparks, T.H., Huber, K. & Croxton, P.J. (2006) Plant development scores from fixed-date photographs: the influence of weather variables and recorder experience. *International Journal of Biometeorology*, **50**, 275–279.

Sparks, T.H., Jeffree, E.P. & Jeffree, C.E. (2000) An examination of the relationship between flowering times and temperature at the national scale using long-term phenological records from the UK. *International Journal of Biometeorology*, **44**, 82–87.

Suarez, A.V. & Tsutsui, N.D. (2004) The value of museum collections for research and society. *BioScience*, **54**, 66–74.